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ON THE INFLUENCES OF GROWTH INHIBITIONS AND COOPERATIVITY INHIBITION ON THE DYNAMICAL BEHAVIOR OF FERMENTATION SYSTEM

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Abstract

Bioethanol is one of many options for renewable fuels that can be a solution for major problems of high energy consumption. This bioenergy is produced via a fermentation process that involves microorganism such as yeast cell. Some problems arising from alcoholic fermentation process are investigated to achieve a very high and competitive performance of bioethanol production by the yeast cell. In this study, we mathematically investigate the effects of

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inhibition and cooperativity of the specific growth rate of yeast cells related to the dynamical behavior of the fermentation system. We model the growth rate of cells using Monod kinetic which adopts the inhibition pattern in enzyme catalyzed reaction rate which is derived as Michaelis-Menten rate law. We assume that there is cooperativity which influences the specific growth rate of cell with n cooperativity order. Analytically, we find that the system exhibits multiple steady states due to the influences of growth inhibitions. Numerically, we find that cooperativity in the cell's growth does not influence the cell's production. However, it influences the production of ethanol along the fermentation process. Low cooperativity produces high ethanol production, and vice versa.

1. Introduction

Bioethanol has been investigated and recommended by many researchers as an important renewable and sustainable alternative fuel source [1-3]. It is produced from agricultural raw material such as sugars (from sugar cane, sweet sorghum, fruits, sugar beets), starches (from grains, root crops, potatoes), and cellulose materials [1, 4]. These raw materials are converted enzymatically by microorganisms to produce bioethanol via fermentation process.

The most used microorganism to produce bioethanol industrially is *Saccharomyces cerevisiae*. It is a yeast cell that has high ethanol productivity such that it can be used in large scale for bioethanol production. Since fermentation is an enzymatic process occurring in the yeast metabolism, several bottlenecks in the metabolic process must be overcome to reach a very high and competitive performance of bioethanol production. Some of these bottlenecks are sensitivity of fermentation system to the environment condition (such pH, temperature, level of oxygen, etc.), inhibition to the growth of the yeast cell, and existence of cooperativity that influences the specific growth rate of the cell [5, 6].

Mathematical models that approximated the dynamical behavior of fermentation system can be classified according to the model of biomass [7, 8]. Segregated model deals with a model that presents biomass as a population of individual cells. While continuum (unsegregated) model presents biomass as a chemical complex in solution. This second model is divided into two models, an unstructured model that regards biomass as one compound which does not vary in composition upon environmental changes, and a structured model that takes into consideration the metabolic and chemical process involved in the fermentation system (such as interactions between chemical compounds and between the biomass and the environment) [7-11]. In this study, we present a mathematical model in terms of an unstructured model that considers biomass as one compound which does not vary in composition upon environmental changes. We take into consideration the growth inhibition not only by substrate and product but also by the density of the yeast cell. The general term of the order of cooperativity is introduced to the model to quantify its effects related to the dynamical behavior and productivity of the fermentation system. We organize the paper as follows: in Section 2, we formulate the unstructured model by involving growth inhibition and cooperativity phenomenon. In Section 3, analytical results are presented to study the steady state condition of the system. Numerical simulation and some discussions are presented in Section 4, and conclusions are presented in the last section.

2. Model Formulation

This section deals with the formulation of the unstructured model of a fermentation system by yeast cell. We assume that the fermentor is a well-mixed system which consists of nutrients (substrate glucose). We model a fermentation system by *Saccharomyces cerevisiae*, a well-known microorganism with high fermentation productivity.

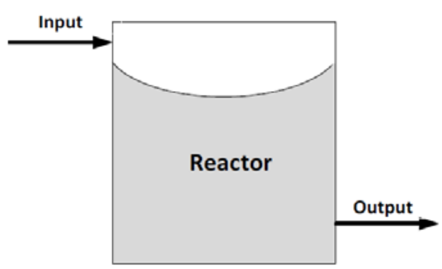


Figure 1. Illustration of bioethanol fermentor.

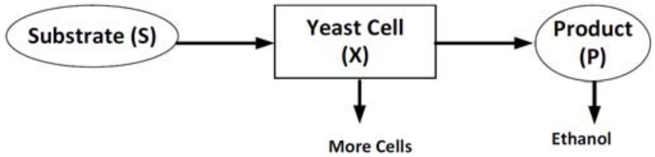


Figure 2. Schematic representation of the external fermentation system.

The specific growth rate of biomass which refers to the glucose uptake is modeled using Monod kinetic [6, 12]:

$$\mu = \mu_m \frac{s(t)}{k_s + s(t)}, \tag{1}$$

where $s(t)$ refers to the concentration of substrate glucose at time t, μ_m is the maximum specific growth rate of the yeast cell, and k_s is the concentration of substrate that will produce a half of maximum specific growth rate, a Monod constant. Due to its similarity with enzyme catalyzed reaction rate which is derived as Michaelis-Menten rate law, Monod kinetic is capable to mimic the exponential growth phase of the yeast cell followed by the decelerating growth phase [12]. Growth inhibitions by glucose and ethanol accumulation also influence the growth of yeast cell such that we have

$$\mu = \mu_m \frac{s(t)}{k_s + s(t)}\left(1 - \frac{s(t)}{s_c}\right)^n \left(1 - \frac{p(t)}{p_c}\right)^n, \tag{2}$$

where s_c and p_c refer to the critical concentration of substrate and product above which cell's growth is completely inhibited, n is the order of cooperativity of the system, $n \geq 1.$ It was reported that more than 25 percent sugar and 15 percent product in the environment of a fermentation system will trigger stress in terms of inhibition during ethanol fermentation [13]. Using a set of ordinary differential equations to describe the dynamic of the fermentor system, we have the following system:

$$\frac{dx(t)}{dt} = \mu x(t)\left(1 - \frac{x(t)}{x_c}\right) - \alpha x(t),$$

$$\frac{ds(t)}{dt} = \alpha Q - q_s x(t) - m_1 s(t) - \alpha s(t),$$

$$\frac{dp(t)}{dt} = q_p x(t) - m_2 p(t) - \alpha p(t), \tag{3}$$

where $q_s = \frac{1}{Y_1}\mu$ and $q_p = Y_2\mu$ are the specific rates of substrate consumption and product formation that is proportional to the specific growth rate of yeast cell and yield coefficients. We assume that there is a maximum population size x_c that environment can sustain the growth of the yeast cells. There is also substrate and product uptake for the maintenance of yeast cell with rate constants m_1 and m_2. Parameter α stands for dilution rate of the fermentation system which describes the flow of medium per unit of time over the volume of culture in a reactor system. In batch cultivation, we have $\alpha = 0$. While in continuous cultivation, fresh medium flows into the system continuously and products are withdrawn at the same flow rate of the inlet flow such that we have $\alpha > 0$. We define initial conditions for the fermentation system as follows: $x(0) = x_0$, $s(0) = s_0$, $p(0) = 0$.

3. Analytical Results

At steady state condition, from the first equation in (3), we obtain $x = 0$ or $\alpha = \mu\left(1 - \frac{x}{x_c}\right)$, with $x \neq 0$. For $x = 0$, we have the first steady state, $T_1 = (x^*, s^*, p^*) = \left(0, \frac{\alpha Q}{(m_1 + \alpha)}, 0\right)$. This steady state will be locally asymptotically stable if it fulfills

$$\mu_m Q\left(1 - \frac{\alpha Q}{(m_1 + \alpha)s_c}\right)^n < (m_1 + \alpha)\left(\frac{\alpha Q}{(m_1 + \alpha)} + k_s\right).$$

If T_1 is a stable steady state, then a washout condition will be produced, i.e., an experimental condition that leads the concentration of yeast cells decreases and vanishes in the reactor.

For $x \neq 0,$ consider the following analysis. From the second equation in (3), we have $x^{**} = (Y_1/\mu)[\alpha Q - (m_1 + \alpha)s^{**}].$ And from the last equation in (3), we get $p^{**} = \dfrac{Y_1 Y_2}{(m_2 + \alpha)}[\alpha Q - (m_1 + \alpha)s^{**}].$ Consider equation $\alpha = \mu\left(1 - \dfrac{x}{x_c}\right).$ By substituting equation (2), we get

$$\mu_m s^{**}\left(1 - \frac{s^{**}}{s_c}\right)^n \left(1 - \frac{p^{**}}{p_c}\right)^n \left(1 - \frac{x^{**}}{x_c}\right) - \alpha(k_s + s^{**}) = 0. \tag{4}$$

Equation (4) together with x^{**} and p^{**} gives a function of $s^{**},$ i.e.,

$$\mu_m s^{**}\left(1 - \frac{s^{**}}{s_c}\right)^n \left(1 - \frac{Y_1 Y_2[\alpha Q - (m_1 + \alpha)s^{**}]}{p_c(m_2 + \alpha)}\right)^n$$

$$\cdot \left(1 - \frac{Y_1[\alpha Q - (m_1 + \alpha)s^{**}]}{\alpha x_c}\right) - \alpha(k_s + s^{**}) = 0. \tag{5}$$

Equation (5) is a nonlinear function of s^{**} which is very difficult to find the closed form of its solutions. Therefore, we will numerically investigate solutions of (5) using parameters in Table 1. For simulation purposes, we will choose one parameter as an independent parameter to show the roots of s^{**} with $n = 2.$ In Figure 3, we can observe the possibilities of the solutions of (5) which depend on the dilution rate as the control parameter. For a certain dilution rate, the system can produce two positive roots of s which generate two positive steady state solutions for system (3). It means that there are possibilities that the system will reveal multiple steady states behavior due to the multiple roots of s.

Table 1. Values of parameter used for simulating model (3)

Parameter	Value	Reference	Parameter	Value	Reference
μ_m	$0.67h^{-1}$	[15]	x_c	$5gl^{-1}$	[15]
Y_1	0.511g/mmol	[14]	s_c	$2000gl^{-1}$	[15]
Y_2	10mmol/g	[14]	p_c	$500gl^{-1}$	[15]
α	$0.027h^{-1}$	[14]	m_1	$1\text{mmol } g^{-1}h^{-1}$	[15]
Q	$280gl^{-1}$	[14]	m_2	$0.5\text{mmol } g^{-1}h^{-1}$	[15]
k_s	$0.28gl^{-1}$	[15]			

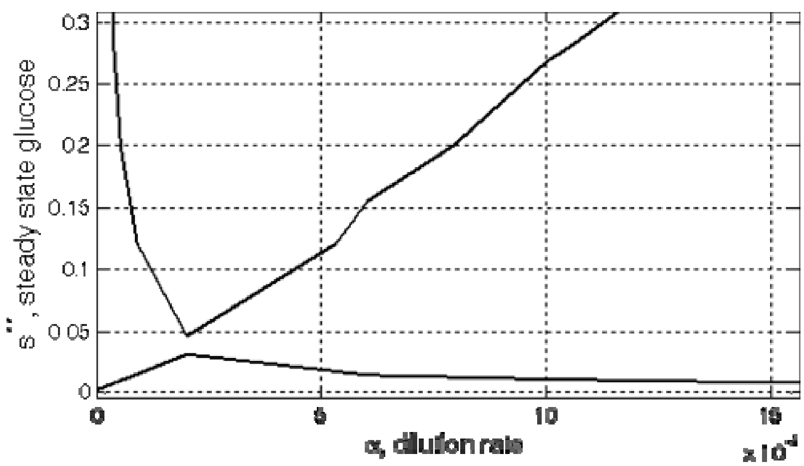


Figure 3. Possibilities of the positive roots of s^{**} for different dilution rates.

Let $T_2 = (x^{**}, s^{**}, p^{**})$, where $x^{**} = \frac{Y_1}{\alpha}[\alpha Q - (m_1 + \alpha)s^{**}]$, $p^{**} = \frac{Y_1 Y_2}{(m_2 + \alpha)}[\alpha Q - (m_1 + \alpha)s^{**}]$ and s^{**} is the positive solution of equation (5). Linearizing system (3) at T_2 yields a linear system with Jacobian matrix:

$$J = \begin{pmatrix} J_{11} & J_{12} & J_{13} \\ J_{21} & J_{22} & J_{23} \\ J_{31} & J_{32} & J_{33} \end{pmatrix},$$

where

$$J_{11} = -\frac{\mu_m s\left(1-\frac{p}{p_c}\right)^n\left(1-\frac{s}{s_c}\right)^n x}{(s+k_s)x_c} + \frac{\mu_m s\left(1-\frac{x}{x_c}\right)\left(1-\frac{p}{p_c}\right)^n\left(1-\frac{s}{s_c}\right)^n}{s+k_s} - \alpha,$$

$$J_{12} = \frac{\mu_m\left(1-\frac{x}{x_c}\right)\left(1-\frac{p}{p_c}\right)^n\left(1-\frac{s}{s_c}\right)^n x}{s+k_s} + \frac{\mu_m s\left(1-\frac{x}{x_c}\right)\left(1-\frac{p}{p_c}\right)^n\left(1-\frac{s}{s_c}\right)^n x}{(s+k_s)^2} - \frac{2\mu_m s\left(1-\frac{x}{x_c}\right)\left(1-\frac{p}{p_c}\right)^n\left(1-\frac{s}{s_c}\right)x}{(s+k_s)s_c},$$

$$J_{13} = -\frac{2\mu_m s\left(1-\frac{x}{x_c}\right)\left(1-\frac{p}{p_c}\right)\left(1-\frac{s}{s_c}\right)^n x}{(s+k_s)\,p_c},$$

$$J_{21} = \frac{\mu_m s\left(1-\frac{p}{p_c}\right)^n\left(1-\frac{s}{s_c}\right)^n x}{(s+k_s)x_c Y_1} - \frac{\mu_m s\left(1-\frac{x}{x_c}\right)\left(1-\frac{p}{p_c}\right)^n\left(1-\frac{s}{s_c}\right)^n}{(s+k_s)Y_1},$$

$$J_{22} = -\frac{\mu_m\left(1-\frac{x}{x_c}\right)\left(1-\frac{p}{p_c}\right)^n\left(1-\frac{s}{s_c}\right)^n x}{(s+k_s)Y_1} + \frac{\mu_m s\left(1-\frac{x}{x_c}\right)\left(1-\frac{p}{p_c}\right)^n\left(1-\frac{s}{s_c}\right)^n x}{(s+k_s)^2 Y_1}$$

$$+\frac{2\mu_m s\left(1-\frac{x}{x_c}\right)\left(1-\frac{p}{p_c}\right)^n\left(1-\frac{s}{s_c}\right)x}{(s+k_s)Y_1 s_c}-\alpha-m_1,$$

$$J_{23}=\frac{2\mu_m s\left(1-\frac{x}{x_c}\right)\left(1-\frac{p}{p_c}\right)\left(1-\frac{s}{s_c}\right)^n x}{(s+k_s)Y_1 p_c},$$

$$J_{31}=-\frac{\mu_m s\left(1-\frac{p}{p_c}\right)^n\left(1-\frac{s}{s_c}\right)^n Y_2 x}{(s+k_s)x_c}+\frac{\mu_m s\left(1-\frac{x}{x_c}\right)\left(1-\frac{p}{p_c}\right)^n\left(1-\frac{s}{s_c}\right)^n Y_2}{s+k_s},$$

$$J_{32}=\frac{\mu_m\left(1-\frac{x}{x_c}\right)\left(1-\frac{p}{p_c}\right)^n\left(1-\frac{s}{s_c}\right)^n Y_2 x}{s+k_s}$$

$$-\frac{\mu_m s\left(1-\frac{x}{x_c}\right)\left(1-\frac{p}{p_c}\right)^n\left(1-\frac{s}{s_c}\right)^n Y_2 x}{(s+k_s)^2}$$

$$-\frac{2\mu_m s\left(1-\frac{x}{x_c}\right)\left(1-\frac{p}{p_c}\right)^n\left(1-\frac{s}{s_c}\right)Y_2 x}{(s+k_s)s_c},$$

$$J_{33}=\frac{2\mu_m s\left(1-\frac{x}{x_c}\right)\left(1-\frac{p}{p_c}\right)\left(1-\frac{s}{s_c}\right)^n Y_2 x}{(s+k_s)\,p_c}-\alpha-m_2.$$

Note that in matrix J, $x = x^{**}$, $s = s^{**}$, $p = p^{**}$. Eigenvalues of J are the solutions of characteristic equation

$$\det(\lambda I - J) = 0$$

or

$$\lambda^3 + a_1\lambda^2 + a_2\lambda + a_3 = 0,$$

where $a_1, \ldots, a_3$ are the coefficients of polynomial that depend on $J_{i,j}$, $i, j = 1, \ldots, 3.$ It has three solutions which can be all real or one real and a complex conjugated pair which forms $\lambda = \eta \pm \sigma i.$ If we have eigenvalues with negative real part, then T_2 will be locally asymptotically stable. While if there is one eigenvalue with positive real part, then T_2 will be unstable. When $\sigma \neq 0,$ we get a damped $(\text{for}\,\eta < 0)$ or an undamped $(\text{for}\,\eta > 0)$ oscillatory behavior after a perturbation.

4. Numerical Results and Discussions

In this section, we simulate system (3) using parameters in Table 1 and the following initial conditions: $x(0) = 1, 3;$ $s(0) = 44;$ $p(0) = 0$ (in g/l). In Figure 4, we can observe that system (3) generates asymptotically stable behavior. All solutions exponentially converge to the positive steady state solution of the system. For the used parameters, this simulation produces high ethanol concentration.

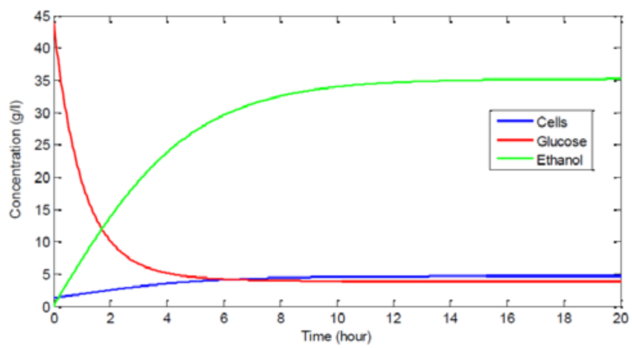
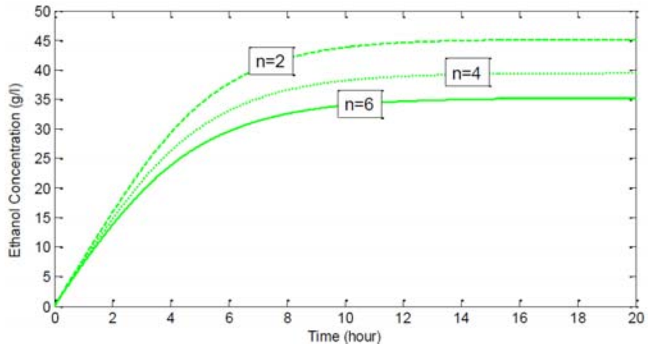
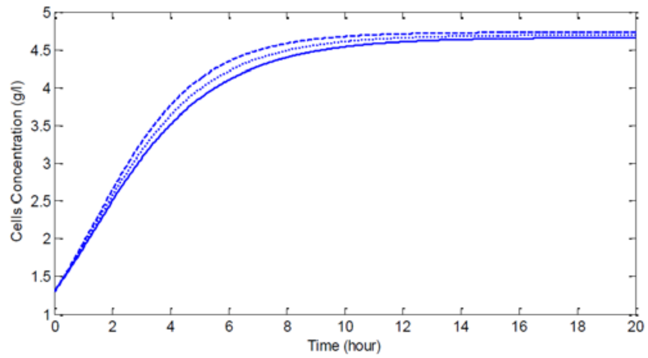


Figure 4. Dynamical observation of system (3) with $n = 2$.

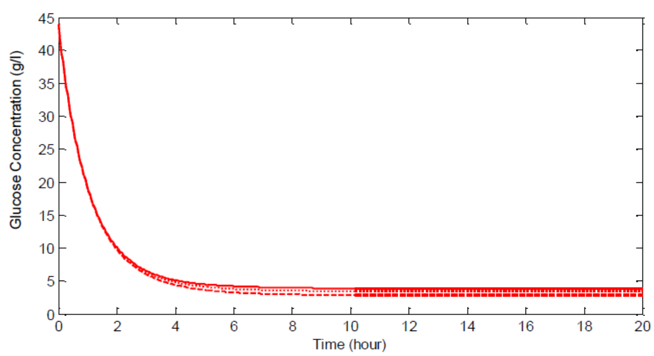
To study the effects of cooperativity on the cell's specific growth rate, in Figure 5, we present simulations of system (3) with different order of cooperativity. We can observe that different cooperativity gives different value of convergence. The fermentation system with low cooperativity gives the highest ethanol production. This cooperativity does not significantly influence the production of new yeast cells since the values of convergence for different orders of cooperativity have small differences.



(a)



(b)



(c)

Figure 5. Dynamical observation of (a) ethanol production, (b) cell's production, and (c) glucose consumption for different order of cooperativity: $n = 2$ (dashed line), $n = 4$ (dotted line), and $n = 6$ (solid line).

5. Conclusions

In this paper, we formulated a mathematical model of fermentation system in terms of an unstructured model. The generated model took into consideration some factors that influenced the growth of yeast cells. These factors also directly influenced the production of ethanol. Analytical results showed that there were possibilities for the system to reveal a washout condition and multiple steady states condition experimentally difficult to overcome. These analytical results indicated that setting of initial experimental conditions, such as initial concentrations of substrates and cells, became a main feature to produce successful and optimal fermentation process. Existence of cooperativity in a fermentation system also became a bottleneck that should be considered in controlling the fermentation system to reach a very high and competitive performance of bioethanol production. Low cooperativity produced high ethanol production, and vice versa.

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