Optimizing Ethanol Production in a Microbial Fermentation Model Using an On-Off Feeding Strategy

Kasbawati[1], A.Y. Gunawan[2,*] , K. A. Sidarto[2], and R. Hertadi[3]

[1]Department of Mathematics, Faculty of Mathematics & Natural Sciences
Jl. Perintis Kemerdekaan Km. 10 Tamalanrea, (Makassar, Indonesia)

[2]Industrial & Financial Mathematics Research Group
Jl. Ganesha No. 10, (Bandung, Indonesia)

[3]Biochemistry Research Division
Jl. Ganesha No. 10, (Bandung, Indonesia)
rhertadi@chem.itb.ac.id

[*]*Corresponding author's email: aygunawan [AT] math.itb.ac.id*

ABSTRACT— ***In a fermentation process, the amount and appropriate timing of feeding nutrient are very essential parameters for achieving efficiency and productivity. In this study, we propose an on-off strategy of feeding glucose for the yeast cell and apply an optimal control theory to find an optimal feeding rate and feeding time to produce a high ethanol production. Using a gradient-based method we solve an optimization problem subjected to a state system constrain and an inequality stability constrain. We find the following feeding strategies: for feeding with shorter delay requires lower glucose supply whereas for longer one it requires higher supply.***

Keywords— fermentation system, optimal control, hybrid gradient-based numerical method

1. INTRODUCTION

Production of biofuel for industrial purposes has currently been the subject of much attention in biotechnology industry. Ethanol is one of biotechnology products which is used primarily as an alternative source to petroleum-based fuels [1]. It is obtained by anaerobic fermentation of sugar-based feedstock as the raw materials by an appropriate microorganism [2, 3]. In recent year, research on improving ethanol production has become a challenge especially to identify the bottlenecks that limit industrial processes and to develop attractive high ethanol performance processes. Since the cost-effective production of ethanol becomes the main goal in identifying the bottlenecks, an effective cultivation method should be developed to generate reactions with high productivity and yield [4].

Many mathematical models have been developed to describe fermentation processes. They consist of unstructured models and structured models which qualitatively incorporate some basic mechanisms of cellular behavior [1, 5, 6]. These models are developed mainly for describing the qualitative behavior of the metabolic system as well as to support an optimal fermentation design.

In the fermentation system, feeding strategy of the raw material is one of the main features since it directly affects the effciency of ethanol production. Substrate-feeding to the growth medium becomes an external control which has an important role in the system. At a low substrate concentration, degradation of glucose is oxidative which leads to lower ethanol production. On the other hand, overfeeding of glucose accelerateS the production of ethanol that simultaneously affects the growth of the yeast cell. This phenomenon is called overflow metabolism [7, 8]. This is a consequence of a saturated oxidative capacity on the level of pyruvate. This metabolic behavior indicates that different substrate-feeding strategies generate different rate of product formation. Therefore an efficient method of feeding nutrients and appropriate time of feeding are very essential parameters for achieving high productivity in the fermentation process. Based on these facts we consider a kinetic model of fermentation reaction to identify an optimal feeding strategy to obtain high ethanol productivity. Since we focus to model the kinetic reaction of a single yeast cell metabolic system, growth inhibition of the cell is not taken into consideration. The kinetic model is derived based on the stoichiometry of biochemical reactions in the central metabolism pathway of a single yeast cell providing the basis of structured modeling. In the previous study (see [9]), we have modeled the feeding process as a continuous process. In the present study we extend our investigation by considering the feeding process as an on-off process, i.e. a combination between zero and non-zero (continuous)

supply. We apply optimal control theory to the fermentation system and use the feeding rate of glucose and the switching instant time between zero and non-zero supply as the control parameters. We use total of ethanol concentrations as the objective in the optimization procedure for this new regulation supply. The main goal of this study is to generate a mathematical model that can adjust and predict the optimal feeding regulation for the yeast cell in producing high ethanol concentration.

We organize the paper as follows. In Section 2, we present the formulation of the kinetic model of fermentation process of a yeast cell. In Section 3, we formulate the optimization problem. In Section 4, we present the computational procedures and numerical simulation results. A summary and some concluding remarks are presented in Section 5.

2. PROBLEM FORMULATION

In this study, a microbial fermentation model of a yeast cell is derived based on the similar assumptions to [9]. A schematic diagram of the central metabolism of the yeast cell is shown in Figure 1. This work follows along the lines of Lei et al. [6], but now for a different metabolic pathway. Fermentation process considered here is assumed a chemical process under ideal fermentation conditions. All chemical conversion processes are catalyzed by different enzymes, and their kinetic equations show the dynamic of single-substrate reactions with irreversible and reversible mechanisms [6, 10, 11]. The kinetic models follow the irreversible and reversible Michaelis-Menten kinetic equations (see [9] for detail about the kinetic equations).

Reaction r_i is catalyzed by the following enzyme (Figure 1): 1. pyruvate kinase, 2. pyruvate carboxylase, 3. pyruvate dehydrogenase complex, 4. pyruvate decarboxylase, 5. alcohol dehydrogenase, 6. acetaldehyde dehydrogenase, 7. acetyl-CoA synthetase, while r_8 refers to the rate of glucose supply and r_9, $\cdots$ r_{12} refer to the product outflows. TCA is the tricarboxylic acid cycle, a key part of aerobic respiration in the yeast cell. The single and the double arrows indicate irreversible and reversible reactions, respectively

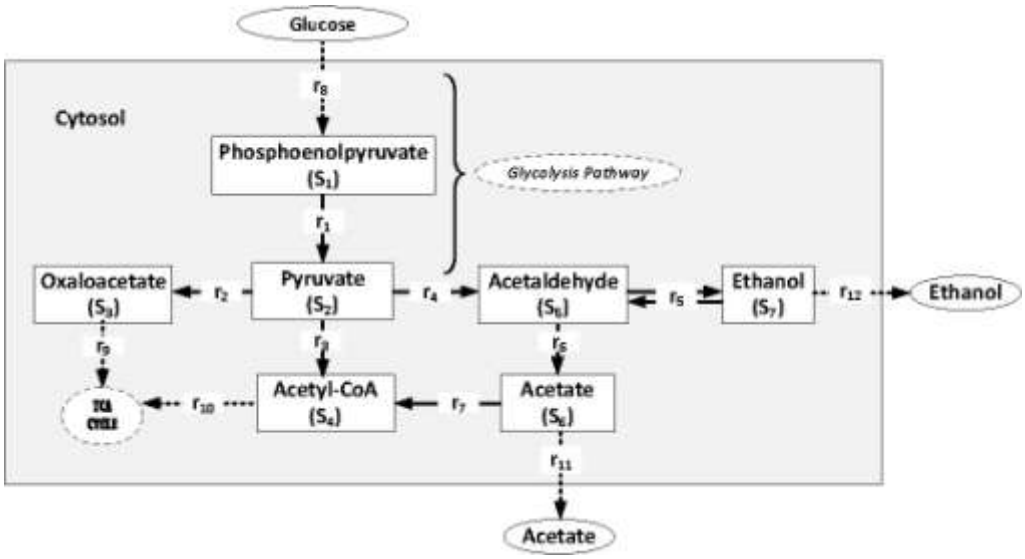

Figure 1: Schematic representation of the central metabolism of *S. cerevisiae*.

Glucose, as the nutrient of the yeast cell; is metabolized via a lumped glycolysis pathway. This process leads to the formation of pyruvate (S_2) from phosphoenolpyruvate (S_1), which is the last intermediate metabolite in the glycolysis pathway. In the previous model (see [9]), we considered a fermentation process with a continuous culture process where the glucose supply was modeled as a continuous constant process $r_8(t) = G$. In the present study, we extend our investigation by considering an on-off feeding rate for the yeast cell. We introduce the feeding function $r_8(t) = u(t)$ given by $u(t)=GH\tau(t)$ defined over $0 \le t \le t_f$ where τ is the switching instant time between zero supply and non-zero supply, t_f is the final time of fermentation reaction, and $H\tau(t)$ is the following Heaviside function

$$H_\tau(\mathrm{t}) = \begin{cases} 0, \text{ if } \quad 0 \le \mathrm{t} < \tau, \\ 1, \text{ if } \quad \tau < t \le \mathrm{t}_f . \end{cases} \tag{1}$$

Therefore, the glucose supply G and the switching instant time τ become the control parameters in optimizing the production of ethanol. Furthermore, in our pathway, we have two metabolite products which are leading to TCA cycle for energy production, and the other products become the extracellular yeast's wastes including ethanol. For the outflow product of the system, it follows the first-order kinetic, $r_i(t)= \delta_k S_j(t)$, $i = 9, \cdots, 12$, $j =3, 4, 6, 7$ with constant δ_k, $k =1,\cdots,4$. Under the above assumptions, the mass balances of all metabolites can be formulated as

$$
\begin{aligned}
\dot{S}_1(t) &= u(t) - \frac{V_1 S_1(t)}{S_1(t)+K_1},\\
\dot{S}_2(t) &= \frac{V_1 S_1(t)}{S_1(t)+K_1} - \frac{V_2 S_2(t)}{S_2(t)+K_2} - \frac{V_3 S_2(t)}{S_2(t)+K_3} - \frac{V_4 S_2(t)}{S_2(t)+K_4},\\
\dot{S}_3(t) &= \frac{V_2 S_2(t)}{S_2(t)+K_2} - \delta_1 S_3(t),\\
\dot{S}_4(t) &= \frac{V_3 S_2(t)}{S_2(t)+K_3} + \frac{V_7 S_6(t)}{S_6(t)+K_7} - \delta_2 S_4(t),\\
\dot{S}_5(t) &= \frac{V_4 S_2(t)}{S_2(t)+K_4} - \frac{V_{5f} K_{5b} S_5(t) - V_{5b} K_{5f} S_7(t)}{K_{5f} K_{5b} + K_{5b} S_5(t) + K_{5f} S_7(t)} - \frac{V_6 S_5}{S_5(t)+K_6},\\
\dot{S}_6(t) &= \frac{V_6 S_5}{S_5(t)+K_6} - \frac{V_7 S_6(t)}{S_6(t)+K_7} - \delta_3 S_6(t),\\
\dot{S}_7(t) &= \frac{V_{5f} K_{5b} S_5(t) - V_{5b} K_{5f} S_7(t)}{K_{5f} K_{5b} + K_{5b} S_5(t) + K_{5f} S_7(t)} - \delta_4 S_7(t),
\end{aligned}
\tag{2}
$$

with initial conditions $S_i(t)= S_{i0}$ (a positive constant) for i =1, ⋯ , 7. Using $t = t_f \tilde{t}$ and $S_i = S_{i0} s_i$ then we have the dimensionless metabolic system,

$$
\begin{aligned}
\dot{s}_1(\tilde{t}) &= \tilde{u}(\tilde{t}) - \frac{v_1 s_1(\tilde{t})}{s_1(\tilde{t})+k_1} = f_1(\tilde{t}),\\
\dot{s}_2(\tilde{t}) &= \frac{v_1 s_1(\tilde{t})}{s_1(\tilde{t})+k_1} - \frac{v_2 s_2(\tilde{t})}{s_2(\tilde{t})+k_2} - \frac{v_3 s_2(\tilde{t})}{s_2(\tilde{t})+k_3} - \frac{v_4 s_2(\tilde{t})}{s_2(\tilde{t})+k_4} = f_2(\tilde{t}),\\
\dot{s}_3(\tilde{t}) &= \frac{v_2 s_2(\tilde{t})}{s_2(\tilde{t})+k_2} - \sigma_1 s_3(\tilde{t}) = f_3(\tilde{t}),\\
\dot{s}_4(\tilde{t}) &= \frac{v_3 s_2(\tilde{t})}{s_2(\tilde{t})+k_3} + \frac{v_7 s_6(\tilde{t})}{s_6(\tilde{t})+k_7} - \sigma_2 s_4(\tilde{t}) = f_4(\tilde{t}),\\
\dot{s}_5(\tilde{t}) &= \frac{v_4 s_2(\tilde{t})}{s_2(\tilde{t})+k_4} - \frac{v_{5f} k_{5b} s_5(\tilde{t}) - v_{5b} k_{5f} s_7(\tilde{t})}{k_{5f} k_{5b} + k_{5b} s_5(\tilde{t}) + k_{5f} s_7(\tilde{t})} - \frac{v_6 s_5}{s_5(\tilde{t})+k_6} = f_5(\tilde{t}),\\
\dot{s}_6(\tilde{t}) &= \frac{v_6 s_5}{s_5(\tilde{t})+k_6} - \frac{v_7 s_6(\tilde{t})}{s_6(\tilde{t})+k_7} - \sigma_3 s_6(\tilde{t}) = f_6(\tilde{t}),\\
\dot{s}_7(\tilde{t}) &= \frac{v_{5f} k_{5b} s_5(\tilde{t}) - v_{5b} k_{5f} s_7(\tilde{t})}{k_{5f} k_{5b} + k_{5b} s_5(\tilde{t}) + k_{5f} s_7(\tilde{t})} - \sigma_4 s_7(\tilde{t}) = f_7(\tilde{t}),
\end{aligned}
\tag{3}
$$

with dimensionless initial conditions $s_1(0) = 1$, $s_i(0) = s_i$ 0 for i > 1, and dimensionless kinetic parameters, $v_i = t_f V_i/S_{10}$, $k_j = K_j/S_{10}$, $k_{5f} = S_{10}/K_{5f}$, $k_{5b} = S_{10}/K_{5b}$, $\sigma_k = t_f \delta_k$, for i, j = 1,⋯, 7 (j = 5), k = 1,⋯, 4. For the on-off glucose supply function $u(t)$, in dimensionless term it becomes

$$\tilde{u}(\tilde{t}) = \tilde{G} H_{\tilde{\tau}}(\tilde{t}), \tag{4}$$

where

$$H_{\tilde{\tau}}(\tilde{t}) = \begin{cases} 0, & \text{if } 0 \le \tilde{t} \le \tilde{\tau}, \\ 1, & \text{if } \tilde{\tau} < \tilde{t} \le 1, \end{cases} \tag{5}$$

with $\tilde{\tau} = \tau / t_f$ and $\tilde{G} = t_f G / S_1^0$. In practice, we restrict ourselves to consider a non-zero upper bound of dimensionless of glucose supply $\tilde{G}_u$. Then in dimensionless term, we define the admissible domain for the feeding rate of glucose and the switching instant time as follows:

$$D = \left\{ (\tilde{G}, \tilde{\tau}) \in \Re^2 \,|\, 0 \le \tilde{G} \le \tilde{G}_u, 0 < \tilde{\tau} < 1 \right\}. \tag{6}$$

3. OPTIMAL CONTROL MODEL

In the section, we formulate the optimization procedure to find the optimal feeding rate and switching instant time for

the feeding process of glucose. We use the total of ethanol concentrations as the objective function to determine whether the supply function $\tilde{u}$ in (4) produces optimal ethanol concentration. Therefore the maximization of ethanol concentration problem can be defined as follows,

$$\max_{\tilde{G}\in D} J = \max_{\tilde{G}\in D} \int_0^1 s_7(\tilde{t},\tilde{G},\tilde{\tau})\, d\tilde{t} \tag{7}$$

for fixed $\tilde{\tau}\in D$. This optimization formulation shows that when the feeding process is delayed about $\tilde{\tau}$, from (7), we may determine a parameter $\tilde{G}$ so thet the ethanol production can be maximized. Regarding the existence and the stability conditions for the positive steady state solution of system (3), in the previous study (see [9]) we have observed that the maximum rate of inflows at all reaction stages should be less then their maximum rate of outflows. This condition will guarantee that the solution of our system will converge to the positive and stable steady state solution. As consequence, there is an inequality constrain on control variables, i.e. $\tilde{u} < v_1$. Introducing a positive variable ϕ, we transform this inequality constrain to become an equality constrain,

$$c(\tilde{u}) = (\tilde{u} - v_1 + \phi) = 0. \tag{8}$$

Let

$$F(s,\tilde{u}) = \begin{pmatrix} (f(s,\tilde{u}) - \dot{s}(\tilde{t},\tilde{G},\tilde{\tau}) \\ c(\tilde{u}) \end{pmatrix}, \tag{9}$$

with $f(s,\tilde{u}) = (f_1,...,f_7)$ and $\dot{s}(\tilde{t},\tilde{G},\tilde{\tau}) = (\dot{s}_1,...,\dot{s}_7)$. Therefore the optimization problem for fixed $\tilde{\tau}, 0 < \tilde{\tau} < 1,$ can be rewritten as follows,

$$\max_{\tilde{G}\in D} J = \max_{\tilde{G}\in D} \int_0^1 s_7(\tilde{t},\tilde{G},\tilde{\tau})\, d\tilde{t}, \quad s.t. \quad F(s,\tilde{u}) = 0, \tag{10}$$

with control variables $p = (\tilde{G},\phi)$ and admissible range

$$\tilde{D} = \left\{ p \in \Re^2 \,|\, 0 \le \tilde{G} \le \tilde{G}_u,\ \varphi > 0 \right\}. \tag{11}$$

To replace the constrain optimization problem (10) into an unconstrain opetimization problem, we introduce a Lagrangian funtional,

$$\mathcal{L}(s,\lambda,p) = \int_0^1 \left\{ s_7(\tilde{t},\tilde{\tau},p) + \lambda^T(\tilde{t}) F(s,\tilde{u}) \right\} d\tilde{t}, \tag{12}$$

with Lagrange multipliers dasdasd. The first-order necessary optimality condition of the optimalization problem (10) states that the optimum is a stationary point of the functional $\mathcal{L}$, $\nabla\mathcal{L}(s,\lambda,p) = 0,$ ∇ where is gradient operator [12]. From $\partial\mathcal{L}/\partial\lambda = 0$ we obtain the metabolic system $\dot{s} = f(s,\tilde{u})$ and constain $c(\tilde{u}) = 0$. From $\partial\mathcal{L}/\partial s = 0$, we get

$$\dot{\lambda}_i = g_i(s,\lambda), \quad \lambda(1) = 0,\ i = 1,...,7, \tag{13}$$

with

$$\begin{aligned}
g_1 &= \frac{v_1 k_1 (\lambda_1 - \lambda_2)}{(k_1 + s_1)^2}, \\
g_2 &= \lambda_2 \sum_{i=2}^{4} \frac{v_i k_i}{(k_i + s_2)^2} - \sum_{i=2}^{4} \lambda_{i+1} \frac{v_i k_i}{(k_i + s_2)^2}, \\
g_3 &= \lambda_3 \sigma_1, \\
g_4 &= \lambda_4 \sigma_2, \\
g_5 &= (\lambda_5 - \lambda_7) \left(\frac{k_{5b} k_{5f} (v_{5f} k_{5b} + v_{5f} s_7 + v_{5b} s_7)}{(k_{5f} k_{5b} + k_{5b} s_5 + k_{5f} s_7)^2} \right) + (\lambda_5 - \lambda_6) \frac{v_6 k_6}{(k_6 + s_5)^2}, \\
g_6 &= (\lambda_6 - \lambda_4) \frac{v_7 k_7}{(k_7 + s_6)^2} + \lambda_6 \sigma_3,
\end{aligned} \tag{14}$$

$$g_7 = -1-(\lambda_5-\lambda_7)\left(\frac{k_{5f}k_{5b}(v_{5b}k_{5f}+v_{5b}s_5+v_{5f}s_5)}{(k_{5f}k_{5b}+k_{5b}s_5+k_{5f}s_7)^2}\right)+\lambda_7\sigma_4.$$

Finally, from $\partial\mathcal{L}/\partial p = 0$, we get

$$\begin{aligned}\frac{\partial\mathcal{L}}{\partial\tilde{G}} &= \lambda_1+\lambda_8 = 0,\\ \frac{\partial\mathcal{L}}{\partial\varphi} &= \lambda_8 = 0.\end{aligned} \tag{15}$$

4. COMPUTATIONAL PROCEDURE AND NUMERICAL RESULTS

In this secrion we present some numerical simulations for the system using kinetic data in Table 1. We assume the final time of reaction is t_f = 24 hours and initial concentration for the first substrate is $S_1(0)$=0.2g/l and zero otherwise. Using the final time of reaction and initial condition for S_1, the kinetic parameters in Table 1 can be normalized.

Table 1: Kinetic parameters for *S. cerevisiae*, reported by Lei et al. (2001) and schomburg et al. (2013). The unit of the kinetic parameters from schomburg et al. (2013) was converted by using their molecular weights

Par	Value	Unit	Ref.	Par	Value	Unit	Ref.
δ_i	0.38	$[h^{-1}]$	[6]	K_6	2.64×10^{-4}	$[g\,l^{-1}]$	[6]
K_1	2.43	$[g\,l^{-1}]$	[13]	V_6	4.8	$[g\,g^{-1}h^{-1}]$	[6]
V_1	63.07	$[g\,g^{-1}h^{-1}]$	[13]	K_{5f}	0.034	$[g\,l^{-1}]$	[6]
K_2	237.5	$[g\,l^{-1}]$	[13]	V_{5f}	2.82	$[g\,g^{-1}h^{-1}]$	[6]
V_2	648	$[g\,g^{-1}h^{-1}]$	[13]	K_{5b}	0.057	$[g\,l^{-1}]$	[6]
K_3	2×10^{-5}	$[g\,l^{-1}]$	[6]	V_{5b}	0.0125	$[g\,g^{-1}h^{-1}]$	[6]
V_3	0.501	$[g\,g^{-1}h^{-1}]$	[6]	K_7	0.0102	$[g\,l^{-1}]$	[6]
K_4	5×10^{-7}	$[g\,l^{-1}]$	[6]	V_7	0.0104	$[g\,g^{-1}h^{-1}]$	[6]
V_4	5.81	$[g\,g^{-1}h^{-1}]$	[6]				

For the case of the on-off supply, the optimization problem can be solved using any optimalization technique such as gradient-based method. In this study, we approximate the optimal solution by using the Steepest Descent (SD) method (see [14] for detail about the SD method). Due to the sensitivity of the SD method to the initial searching step, here we use a population based approach to determine the best initial guess for the SD method. Using Sobol Sequence, we generate initial population randomly. This method will ensure that the population is generated randomly in all area of the domain search (for detail about the method see for example [15]). Subsequently, the centers of the population are determined using clustering technique, and then the best individual is chosen to become the initial guess fo the SD method. Here, we use c-means fuzzy clustering with Xie-Beni to determine number of cluster (see for example [16,17,18] for detail about the method). The computational produce is given as follows:

(1) Set the switching time $\tilde{\tau}\in(0,1)$ and initial step size $\beta_0 > 0$.

(2) Randomly generate an intial population for $p\in\tilde{D}$ and $\lambda_8\in\Re$ with a number of individuals using the following equation,

$$x = x_l + r(x_u - x_l), \tag{16}$$

where r is a quasi-random parameter which is generated from [0,1] using Sobol Sequence and $x_{l(u)}$ is the lower (upper) bound vector of the optimization variables. Here the upper bound of glucose supply is taken from [19], i.e. $G_u = 7.5\,gl^{-1}h^{-1}$. In dimensionless term we have $\tilde{G}_u = 900$.

(3) Cluster the population to find the centers.

(4) Evaluate the objective function J on the centers and find the best individual.

(5) Set the best individual as the initial guess for the SD method.

(6) Solve system (3) using a forward Runge Kutta method.

(7) Compute the actual objetive function J.

(8) Solve system (13) using a backward Runge Kutta method.

(9) Calculate the gradient equations in (15).

(10) Update x using the following equation,

$$x_{k+1} = x_k + \beta_k h_{sd}, \tag{17}$$

where $\beta_k = \left(\beta_0 / 2^k\right)$ is the step size which is adapted in every iteration and h_{sd} is the normalized gradient of $\mathcal{L}$ at x_k,

$$h_{sd}(x_k) = \frac{\nabla \mathcal{L}(x_k)}{\left\|\nabla \mathcal{L}(x_k)\right\|}. \tag{18}$$

(11) Return to step 6, unless termination criteria is satisfied.

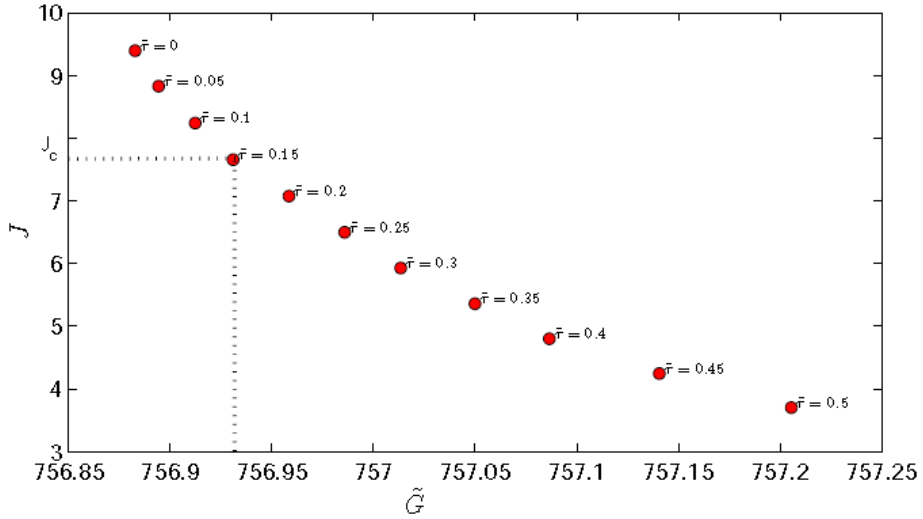

Figure 2: The scatter plot of the optimal total of dimensionless ethanol concentrain *J* with respect to the optimal dimensionless glucose supply $\tilde{G}$ for fixed dimensionless switching instant time $\tilde{\tau}$.

By applying these numerical procedures for different switching instant time $\tilde{\tau}$, we get the scatter plot of the total ethanol concentration with respect to the optimal glucose supply which is depited in figure 4. We observe that total of ethanol concentration resulted from the on-off supply does not give the highest prodution compared to the continuous one (no delay). However, when the feeding supply is delayed about $\tilde{\tau}$ time, the results provide some alternative ways in determining how much the feeding rate should be set to attain an optimal ethanol production. For instance, when the feeding process is delayed about $\tilde{\tau} = 0.1$, the fermentation process will achieve the optimal ethanol production when the nutrient is supplied about $\tilde{G} = 756.92$. Furthermore, we remark that experimental data from [6,19], *G*=5.7 g/l $\left(\tilde{G} = 684\right)$ produces total of dimensionless ethanol concertration for continuous supply, $J_C = 7.72$ (see Figure 4). According to our model, it does not give a maximum ethanol production. The similar result can be obtained by delay the feeding process about $\tilde{\tau} = 0.15$ and set the glucose supply at $\tilde{G} = 756.93$. It indicates that by applying these regulation rules, we can reduce total of glucose supply used along the on-off fermentation system about 5.94 % of the total of glucose supply used in the continuous fermentation system. So does for the feeding processing time which can be shortened about 15% of the continuous fermentation time.

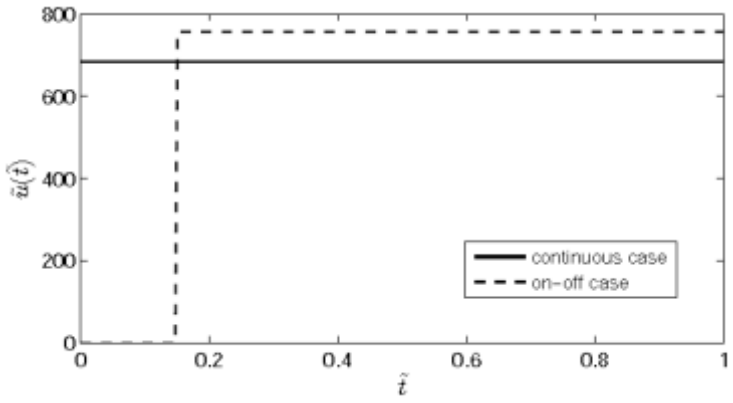

Figure 3: Comparison of glucose supply using to ontinuous method (solid line) and the on-off method (dashed line)

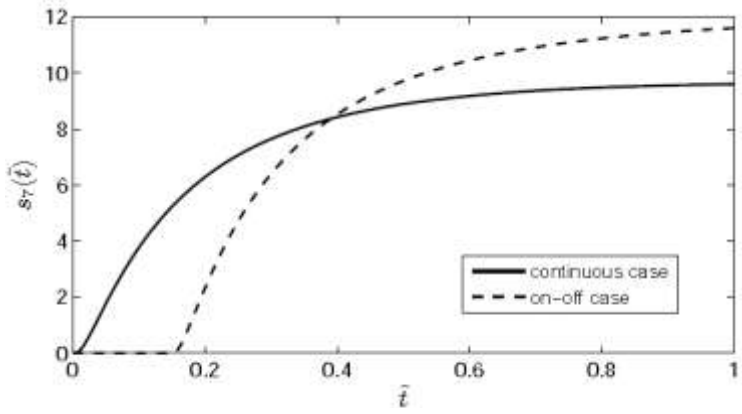

Figure 4: Comparison of ethanol production resulted from the continuous supply (solid line) and the on-off supply (dashed line).

5. CONCLUSIONS

In this paper we investigated a kinetic model of fermentation system of single yeast cell. We modeled the feeding process as an on-off feeding process as the external control to find the optimal supply for fixed time of supply. We used total of ethanol concentration as the objective in the optimization process. We found that for a certain delay of feeding process there was a certain supply in which the production of ethanol attained an optimal solution. The longer feeding process, the greater feeding supply was. Our results may be used as a guidance in the experiment to decide which is the appropriate feeding rule in the fermentation process such that the optimal desired product can be obtained.

6. ACKNOWLEDGEMENT

The authors would like to thank the anonymous reviewers for their valuable comments and suggestions which led to improvements in the quality of the paper. The first author is also grateful to Dr, Loeky Haryanto for his fruitful comments. This work was supported by Riset KK ITB No. 1064y/ I1.C01.2/ PL/ 2014.

7. REFERENCES

[1] I.C.P. Astudillo and C.A.C. Alzate, "Review: Importance of stability study of continuous systems for ethanol production", Journal of Biotechnology, vol. 151, pp. 43–55, 2011.
[2] F.W. Bai, W.A. Anderson, and M. Moo-Young, "Ethanol fermentation technologies from sugar and starch feedstocks", Biotechnology Advances, vol. 26, pp. 89–105, 2008.
[3] L. Laopaiboon, P. Thanonkeo, P. Jaisil, and P. Laopaiboon, "Ethanol production from sweet sorghum juice in batch and fed-batch fermentations by *Saccharomyces cerevisiae*", World J. Microbiol Biotechnol, vol. 23, 1497-1501, 2007.
[4] J. Lee, S. Y. Lee, S. Park, and A. P.J. Middelberg, "Control of fed-batch fermentations", Biotechnology Advances, vol. 17, pp. 29–48, 1999.
[5] A. A. Esener, T. Veerman, J. A. Roels, and N. W. F. Kossen, "Modeling of bacterial growth; formulation and evaluation of a structured model", Biotechnol. Bioeng., vol. 24, pp. 1749–1764, 1982.
[6] F. Lei, M. Rotboll, and S.B. Jorgensen, "A biochemically structured model for *Saccaromyces cerevisiae*", Journal of Biotechnology, vol. 88, pp. 205–221, 2001.

[7] H. T. B. Pham, G. Larsson, and S.O. Enfors, "Growth and energy metabolism in aerobic fed-batch cultures of *Saccharomyces cerevisiae*: Simulation and model verification", Biotechnology and Bioengineering, vol. 60, pp. 1097–0290, 1998.
[8] B. Sonnleitner and O. Kappeli, "Growth of *Saccharomyces cerevisiae* is controlled by its limited respiratory capacity: Formulation and verification of a hypothesis", Bio. & Bioeng., vol. 28, pp. 927–937, 1986.
[9] Kasbawati, A. Y. Gunawan, R. Hertadi, and K. A. Sidarto, "Effects of time delay on the dynamics of a kinetic model of a microbial fermentation process", the ANZIAM J., vol. 55, no. 4, pp. 336–356, 2014.
[10] D.L. Nelson and M.M. Cox, Lehninger: Principles of biochemistry, Whfreeman, Madison, 4 edition, 2005.
[11] J.T. Pronk, H. Yde Steensma, and J.P. Van Dijken, "Pyruvat metabolism in *Saccharomyces cerevisiae*", Yeast, vol. 12, pp. 1607-1633, 1996.
[12] F. L. Lewis, Optimal control, A Wiley-Interscience publication, 1986.
[13] I. Schomburg, A. Chang, S. Placzek, C. Shngen, M. Rother, M. Lang, C. Munaretto, S. Ulas, M. Stelzer, A. Grote, M. Scheer, and D. Schomburg, "Brenda in 2013: integrated reactions, kinetic data, enzyme function data, improved disease classification: new options and contents in Brenda", Nucleic Acids Res., vol. 41 (Database issue), pp. D764-772, 2013.
[14] D. Goldberg, Genetic Algorithm in Search, Optimization, and Machine Learning, Addison-Wesley, New York, 1989.
[15] S. Burhenne, D. Jacob, and G. P. Henze, Sampling based on Sobol sequences for Monte Carlo techniques applied to building simulations, In Proceedings of Building Simulation 2011, the 2th Conference of International Building Performance Simulation Association, Sydney, 2011.
[16] J. C. Bezdek, Pattern Recognition with Fuzzy Objective Function Algorithms, Springer Publishers, New York, 1981.
[17] J. C. Bezdek, R. Ehrlich, and W. Full, "FCM: The fuzzy c-means clustering algorithm", J. Computers & Geosciences, vol. 10, pp. 191–203, 1984.
[18] R. Duda and P. Hart, Pattern classification and scene analysis, Wiley-Interscience, New York, 1973.
[19] E. Postma, C. Verduyn, W. A. Scheffers, and J. P. van Dijken, "Enzymic analysis of the crabtree effect in glucose-limited chemostat cultures of *Saccharomyces cerevisiae"*, Applied and environmental microbiology, vol. 55, no. 2, pp. 468–477, 1989.