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IOP Conf. Series: Journal of Physics: Conf. Series **890** (2017) 012050 doi:10.1088/1742-6596/890/1/012050

A mathematical model for ethanol fermentation from oil palm trunk sap using *Saccharomyces cerevisiae*

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Abstract. This paper presents a mathematical model and solution strategy of ethanol fermentation for oil palm trunk (OPT) sap by considering the effect of substrate limitation, substrate inhibition product inhibition and cell death. To investigate the effect of cell death rate on the fermentation process we extended and improved the current mathematical model. The kinetic parameters of the model were determined by nonlinear regression using maximum likelihood function. The temporal profiles of sugar, cell and ethanol concentrations were modelled by a set of ordinary differential equations, which were solved numerically by the 4th order Runge-Kutta method. The model was validated by the experimental data and the agreement between the model and experimental results demonstrates that the model is reasonable for prediction of the dynamic behaviour of the fermentation process.

1. Introduction

The economic growth and production of goods effect in energy crises and environmental pollution. In the last century the energy consumption has increased to 17-fold with the present rate of energy consumption, it is predictable that the world's oil reservoir will be diminished by 2050 [1]. The combustion of fossil fuels leads to greenhouse effect and global warming. These issues remind us the need to find alternative fuel resources which are renewable and counted as eco-friendly. In Malaysia, palm oil tree has become the main source of biomass to produce renewable bioenergy. The conversion of biomass to ethanol involves a large number of physical and chemical transformations. The chemical properties of biomass material are complex and the reaction kinetics for the degradation of biomass is not well understood [2]. For this reason in order to understand, to operate, to optimize and to control ethanol fermentation processes, a more complete knowledge of dynamic behavior is required. An appropriate kinetic model of ethanol fermentation would be a powerful instrument for increasing fermentation efficiency and process optimization [3].

Fermentation kinetic model is an important tool to describe the yeast behavior, metabolism and bioethanol regulation. The growth of microbial cell can be described by the structured and unstructured model during fermentation. The unstructured models describe microbial kinetics include the most fundamental observations involving microbial growth processes like biomass concentration, substrate consumption and produce metabolic products [4]. They can be used to describe the bioprocess under various operating conditions of temperature, pH, and other adjustable variables. Compared to the unstructured kinetic models, structured models is usually complex to estimation of kinetic parameters, mainly because of nonlinearities, the large number of parameters, and interactions

among complex microbial systems at the molecular level such as DNA, RNA, protein etc. For this reason, relatively simple unstructured kinetic models such as the Monod model and Luedeking–Piret (LP) model have frequently been used for practical application. Hence an unstructured comprehensive kinetic model was proposed in this study that is modified from the Monod kinetics which responds to the changes in the environmental conditions. To our knowledge, no investigations have been carried out on the unstructured model for ethanol fermentation from oil palm trunk sap. In this study, experimental data from batch fermentations of ethanol from the OPT sap using *Saccharomyces cerevisiae* were examined in order to form the basis of kinetic model of the process.

A number of models have been proposed by Esfahanian, Rad [5]; Zhu, Fang [6]; and Liu and Li [3] of batch alcoholic fermentation kinetics. The main factors such as substrate limitation, substrate inhibition, product inhibition and cell death govern the fermentation kinetics, but none of these models accounts those kinetic factors simultaneously. In addition, Phisalaphong, Srirattana [7] studied the inhibition effect of both substrate and product with death rate for ethanol production by cane molasses. Therefore, in this study we have focused firstly to extend and improve the current Oliveira, Oliveira [8] mathematical model that thoroughly described the kinetics of cell activities on the OPT sap from the beginning up to the stationary phase in order to maximize the production of ethanol. Secondly, the parameters in the kinetic expressions were determined; this was achieved by measuring the concentrations of cells, product and substrate as they vary over time and then using nonlinear regression methods to estimate the kinetic parameters.

2. Mathematical Modelling

In order to effectively analyze the kinetics of the fermentation process, we need to understand the ethanol production route and then the phenomenon to express in terms of mathematical equations. The proposed model extended Oliveira's model [8] by adding cell death rate based on the following assumptions:(a) Limitation of yeast growth by shortage of substrate (b) Inhibition of yeast growth by ethanol and substrate (c) Cell death or inactivation does exist.

To construct a mathematical model of ethanol fermentation by *S. cerevisiae Kyokai no.7*, a comprehensive kinetic model modified from the Monod kinetics $[\mu=\mu_{\max}S/(KS+S)]$ [9] responding to changes in the culture conditions was used. Monod equation is widely used to describe cell growth. The equation defines the relation between the growth rate and the substrate concentrations. In our model from Oliveira, Oliveira [8], the specific growth rate, μ of the microorganism can be described by the modified Monod equation that includes substrate inhibition and product inhibition as follow:

$$\mu = \frac{\hat{\mu}S}{K_S + S + \frac{S^2}{K_i}}\left(1-\frac{P}{P_{\max}}\right)^n \tag{1}$$

where P is ethanol concentration, S is substrate concentration, $\hat{\mu}$ is the maximum specific growth rate, K_S is the substrate inhibition constant for cell growth, K_i is the inhibition parameter for sugar, and $P_{\max}$ is the inhibition parameter for ethanol. The important features of the equation (1) is that the growth rate will be zero when substrate concentration is too small $(S << K_S)$ and when the substrate concentration is too high $(S >> K_S)$ the specific growth rate will be maximum.

The rate of cell growth, ethanol production and substrate consumption were related to the cell concentration (X), ethanol concentration (P) and substrate concentration (S) as follows:

$$\frac{dX}{dt} = \mu X - K_d X \tag{2}$$

$$\frac{dS}{dt} = -\frac{\alpha}{Y_{P/S}}\mu X \tag{3}$$

$$\frac{dP}{dt} = \alpha\mu X \tag{4}$$

The parameter $Y_{P/S}$ is the yield coefficient for ethanol on substrate used for ethanol formation, K_d is the cell death rate, α is the model parameter, and *n* is a constant. A substrate such as glucose is used to form cell material and metabolic products as well as the maintenance of cells [10]. According to our model, the ethanol production rate depends on instantaneous biomass (cell) concentration, *X*.

The ordinary differential equations (2)-(4) were solved numerically by the 4th order Runge-kutta (RK4) Method in which the values of the dependent variables (X, P, S) at any time (t) were calculated by series of small steps from the initial values and were solved using the MATLAB.

3. Results and Discussion

3.1. Parameters estimation

Parameter estimation is an essential part in the authentication and consequential use of a mathematical model [11]. The unknown parameters for the proposed mathematical model in Eq. (2), (3) and (4) were estimated using the experimental data obtained from the batch fermentations. The initial values of sugar and cell were obtained from the experimental results with average values of $S(0) = 86.63g/L,\ X(0) = 1.25g/L$ respectively. Inhibition parameter for ethanol, $P_{\max}$ also obtained from the experimental results for different temperatures and ethanol toxic power, n was obtained from the literature [8]. Since our model was a non-linear model with multi-parameters, the optimization for parameter set significantly depended on the initial guesses for the parameter values [7]. Hereby, the initial parameter values $\hat{\mu}$, K_d , K_S , $Y_{P/S}$, K_i , α were tentatively estimated by manual adjustment to obtain a good fit to the experimental data. Then the initial guesstimates of kinetic parameters were re-calculated by running the program iterations. The best–fit values of the parameter were determined by the least-squares method based on nonlinear regression function performed in MATLAB. Table 1 summarizes the estimates of the kinetic parameters.

Table 1. Values of the kinetic parameters at different temperature estimated by nonlinear regression.

Parameter	Notation	Estimated value		
		25°*C*	30°*C*	35°*C*
Maximum specific growth rate (h^{-1})	$\hat{\mu}$	0.7021	0.5557	0.4950
Substrate inhibition constant for cell growth (g/L)	K_S	0.0162	0.0191	0.5520
Specific cell death rate (h^{-1})	K_d	0.0021	0.0032	0.0021
Apparent yield coefficient for substrate to ethanol conversion	$Y_{P/S}$	0.6506	0.5820	0.5601
Inhibition parameter for sugar (g/L)	K_i	115.1792	243.95	148.31
Inhibition parameter for ethanol (g/L)	$P_{\max}$	29.9024	30.7600	26.0600
Model parameter (g/g)	α	0.7598	0.6212	0.6701

3.2 Model prediction and validation

The initial conditions at the start of the fermentation process were as follows $S(0) = 86.63g/L, X(0) = 1.25g/L$ at different temperature. The apparent (or overall) maximum specific growth rate could vary from 0.10 to 0.78 h^{-1}depending on yeast strains and operating conditions [12]. The estimated value of $\hat{\mu}$ was obtained from the model. As for the cause of the inhibition effect on cell growth, a high temperature could result in changing the transport activity or saturation level of soluble compounds and solvents in the cells, which might increase the accumulation of toxic concentration including ethanol inside cells. The lowest value of K_S happened at 25 ºC indicates a high attraction of the microorganism for the substrate utilization. The highest value of the inhibition parameter for sugar (K_i =243.95) at 30 ºC indicate that the ethanol was inhibited by the substrate after that level of concentration. Therefore, the ethanol inhibition parameter value $P_{\max}$ =30.76 was the most at 30 ºC. The estimated values of $Y_{P/S}$ are near to the experimental values.

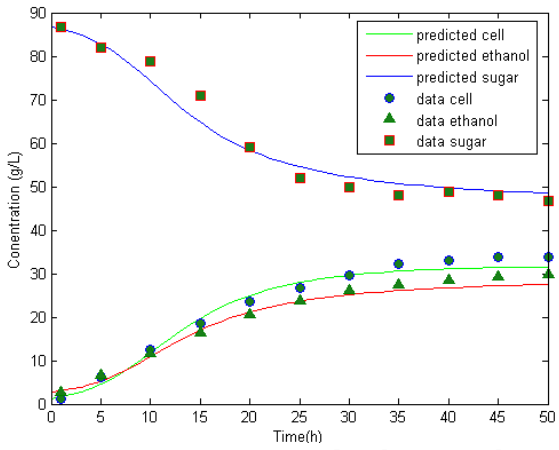

Figure 1. Experimental data and model predictions of batch fermentation at $25°C$ temperature.

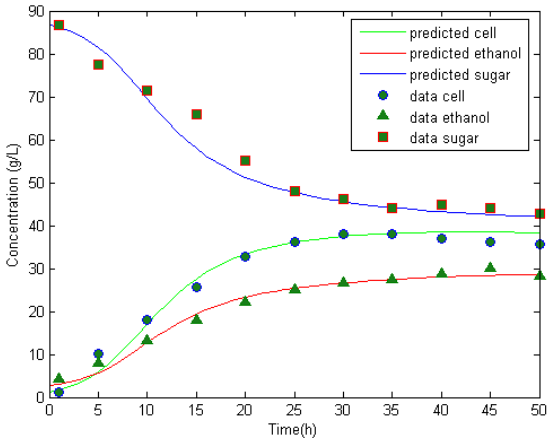

Figure 2. Experimental data and model predictions of batch cultivations at $30°C$ temperature.

From the Figure.1, it can be seen that typical transient concentration profiles i.e. the substrate concentration reduced monotonically with time until its full reduction, while cell and product concentrations increased monotonically until a stationary phase was attained. Allowing to this Figure, model predictions (lines) agree adequately with the experimental data (circles) qualitatively. According to the Figure. 2, the results of the model and the experimental data consistently interpreted that the predicted models gave a satisfactory fit to the experimental data qualitatively for the cell, ethanol and substrate concentration at 30 ºC temperature from the beginning up to the stationary phase was observed. From the Figure. 3, the predicted result detected that the models of the cell and ethanol a slight departed at the end of the fermentation due to the incoherent of the cell growth at the high temperature.

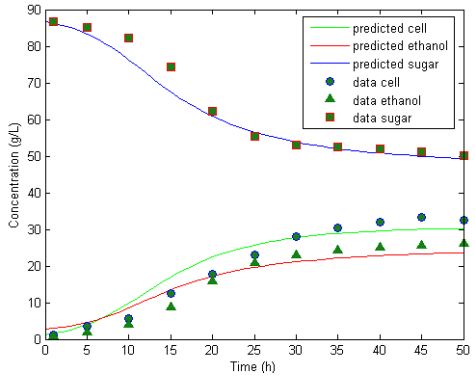

Figure 3. Experimental data and model predictions of batch cultivations at 35°*C* Temperature.

Table 2. relative root mean squared error (rRMSE) for accuracy of the proposed model.

Profile	rRMSE%		
Temperature	25 °C	30 °C	35 °C
Cell (*X*)	2.2818	12.5239	34.0691
Sugar (*S*)	12.3484	7.5916	7.2208
Ethanol (*P*)	8.0751	26.8538	48.0362

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Profile	rRMSE%		
Temperature	25 °C	30 °C	35 °C
Cell (*X*)	3.6520	35.5699	34.3893
Sugar (*S*)	5.5635	10.2946	14.0975
Ethanol (*P*)	44.9484	22.8361	4.3297

3.3 Model performance

The model parameters were approximately estimated by the initial guesstimate and then adjusted using a non-linear regression technique assisted by MATLAB to minimize the sum-of-squares deviation between the model predictions and experimental data. Table 2 shows the Relative Root Mean Squared Error (rRMSE) analysis at different temperature for sugar, cell, and ethanol profiles. Relative root mean squared error (rRMSE) was used for model accuracy. The values are the means with the corresponding of confidence intervals (95%). The relative root mean square error (rRMSE) was calculated using the following equation:

$$rRMSE = 100 \times \frac{1}{\overline{A}} \sqrt{\frac{1}{n} \sum_{r=1}^{n} (O_r - P_r)^2} \quad (5)$$

where A is the average observed value, n is the number of samples, O_r is the observed value of profile r, and P_r is the predicted value of the profile r.

In our model at all temperatures, there was a good agreement between the measured and predicted data with rRMSE values for the cell growth and substrate consumption. This result has been predicted with the consideration of the cell death rate. Therefore, it could be concluded that cell death rate has a very significant influence on the mathematical study of the ethanol production through fermentation process. However, the RMSE values of the ethanol production profiles for the temperature 30°C and 35°C were greater than 15% with the values range from 26.8538% and 48.0362% due to some differences in fermentation and uncontrolled conditions of the model. Even the error is high for ethanol, the model matched with the experimental data qualitatively.

4. Conclusion

Fermentation is a very complex process, and it is often very difficult to obtain a complete picture of what is actually going on in a particular fermentation. To increase the accuracy of the model, the parameter estimation function should depend on cell death. An acceptable agreement was obtained from our proposed model with the experimental data when considered the cell death rate. The extended model improved the predictive capabilities of the dynamic behavior, hence increased our understanding on fermentation process. Cell death rate plays a significant role in the mathematical models and should be included in formulating a fermentation process, which can serve as guidance to further optimize the ethanol fermentation process.

Acknowledgements
This research is supported by the research grant RDU150399, Universiti Malaysia Pahang, Malaysia.

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