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Study of growth kinetic and modeling of ethanol production by *Saccharomyces cerevisae*

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There is a growing interest in bioethanol as biofuel since it has the possibility to be the potential substitute for fossil fuels. Ethanol batch fermentation of *Saccharomyces cerevisiae* strain was carried out in 10 L stirred tank bioreactor for 72 h at 0.075 vvm of aeration and 75 rpm of agitation speed. 85.8% conversion efficiency of ethanol production from glucose substrate was accomplished. This study investigated the *S. cerevisiae* growth kinetics and ethanol productivity using computer simulation of four different kinetic models which are: Monod, Contois, Modified Monod and Teisser. Teisser model gave marginally better fit than other models tested as it obtained the highest correlation coefficient (0.96299). Based on Leudking-Piret model, it could be concluded that ethanol batch fermentation is a non-growth associated process.

Key words: Kinetic parameters, simulation, cell growth, ethanol, *Saccharomyces cerevisiae*.

INTRODUCTION

In recent years, there has been extensive research of alternative fuels as replacement for fossil fuels in order to fulfill the world's energy demand. The enhancement of alternative energy production is invigorated by the oil reserves depletion, the global climate change, the increase in oil prices and the sense of energy independence and security (Drapcho et al., 2008). Bioethanol and the rest of other biofuels offer more advantages than fossil fuels since it provides renewable and sustainable sources of energy. The implementation of bioethanol has become gradually more appealing in the present day as bioethanol has the possibility of being applied in transportation and electricity generation. The use of bioethanol as gasoline oxygenate is beneficial in terms of higher oxygen content, octane number and reduction of CO emission (Cardona et al., 2010). Furthermore, E10 (10% ethanol, 90% gasoline) application needs no engine modification to vehicle (Mousdale, 2008). Moreover, it has been broadly employed in some countries, specifically USA where corn is the main feedstock and Brazil with sugarcane as the raw material for ethanol production (Bourne, 2005; Mousdale, 2008).

Feedstock of bioethanol ranges from agriculture residues, virgin biomass, waste paper, organic fraction of municipal solid waste (MSW) to other materials containing fermentable sugar (McMillian, 1997). The raw materials as the carbon substrate that are not in the form of reducing sugar need to undergo hydrolysis process prior to fermentation (Torres and Baratti, 1988; Elshahed, 2010). Agricultural waste such as rice husk, empty fruit bunch (EFB) from palm oil and sago residue from Sarawak has high potential to be the source of bioethanol production in Malaysia due to their abundance. The most commonly employed methods for bioethanol generation are fermentation using *Saccharomyces cerevisiae*, the baker's yeast and bacterial fermentation by *Zymomonas mobilis* (Abd. Aziz, 2002; Elshahed, 2010). Bioreactor fermentation provides feasibility to control the physical factors such as aeration, agitation, temperature, pH and pressure for optimum bioethanol yield. Aeration is an essential factor for *S. cerevisiae* fermentation even though yeast has the ability to grow under anaerobic condition (Cardona et al., 2010). Agitation is entailed during fermentation in order to warrant efficient nutrient transfer to the cell surface (Doran, 1997; Lee, 2008).

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Unstructured kinetic model considers cell as a uniform quantity without internal dynamic (Arellano-Plaza et al., 2007) since cell growth involves various biochemical networks and chemical reaction (Lee, 2008). On the contrary, structured kinetic models are based on the biomass components, specifically concentration of DNA, RNA, protein, metabolism and enzymes (Cinar et al., 2003).

The study by Nanba et al. (1997) proposed *S. cerevisiae* kinetic model of ethanol batch fermentation based on enzyme deactivation kinetic. It is owing to their discovery that lower culture temperature caused slower growth and ethanol production, yet the final cell mass and ethanol concentration are higher than those for higher temperature culture.

The growth kinetic models of Malthus, Monod and Logistic were tested for the effect of various carbon substrates on the growth kinetics and ethanol productivity of *S. cerevisiae* strain PTCC 24860 (Shafaghat et al., 2009). From the study, Monod model was used to fit the growth kinetic data since it had obtained the highest maximum specific growth rate (μ_{max}).

The cell growth and ethanol production of respiration-deficient yeast mutant via anaerobic growth on a solid medium is estimated by a growth-model associated with CO_2 evolution rate (Sato and Yoshizawa, 1988).

In this study, batch ethanol fermentation of glucose by *S. cerevisiae* was carried out by using 10 L bioreactor. The objective of this study was to investigate the growth kinetic of batch ethanol fermentation. This work also aims to identify the best fit model from the literature to represent batch ethanol fermentation using computer simulation. The simulation was performed through the application of *ode15s* function of MATLAB.

MATERIALS AND METHODS

A dried form of industrial *S. cerevisiae* yeast was used in this research. For inoculum, 100 ml of distilled water was heated to 40°C in a shake flask and 0.5% (w/w) of *S. cerevisiae* yeast was added to the warm water to activate the yeast. The mixture was left for 5 to 10 min at 150 rpm. The inoculums size was set to have a cell concentration of 5.3×10^7 cells per ml. Dilution of the inoculums was done when the concentration of the cells was too high. One gram of yeast contained 25 billion of cells (25 billion/g yeast), thus the initial cell concentration was 2.12 g/L.

Fermentation

0.5% (w/w) of urea and 0.05% (w/w) of NPK (nitrogen, phosphorus and potassium) was added to the 10 L bioreactor with eight liters of working volume (Nadir et al., 2009). The media was set to have 20% (w/v) glucose concentration. After 10 min, the activated yeast solution was added to the bioreactor. The mixture was mixed well for 5 min. Then, the agitation and the aeration were change to 75 rpm and 0.075 vvm, respectively until 72 h of incubation.

Analyses

Sampling was done for every six hours with 15 ml of sample collec-

ted for the measurement of glucose and ethanol concentrations and centrifuged at 5000 rpm for 15 min at 4°C to remove the cell. The supernatants left were then analysed for ethanol and glucose concentration using GC/MS and FTIR, respectively.

Mathematical modeling

Monod model

The most widely utilized unstructured kinetic model is Monod model given by (Bailey and Ollis, 1986):

$$\mu = \frac{\mu_{max}\, S}{K_S + S} \tag{1}$$

Where, μ is the specific growth rate (h⁻¹), S is substrate concentration (g/L), K_s is Monod constant and μ_{max} is defined as growth rate. It has been observed that Monod model has a significant similarity with the Michaelis-Menten kinetics (Doran, 1997; Dunn et al., 2003). Culture that grows on single substrate can be described by Monod model. Substrate in Monod model is known as growth-limiting substrate due to the dominant influence of a single substrate (Doran, 1997; Drapcho et al., 2008). Batch fermentation mass balance for biomass generation rate, substrate consumption rate and product formation rate can be illustrated respectively as follows (Doran, 1998; Lee, 2008):

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

$$\frac{dS}{dt} = -\frac{\mu\, X}{Y_{XS}} \tag{3}$$

$$\frac{dP}{dt} = q_p X \tag{4}$$

Where, X is the biomass concentration (g/L); Y_{XS} is the biomass yield (g biomass/g glucose); P is the product concentration (g/L) and q_p is the specific product formation rate (h⁻¹).

The kinetic parameter such as μ_{max} was calculated during the exponential phase from the slope of the graph of ln X vs time (Dunn et al., 2003). K_s value is the concentration of substrate when μ is equivalent to half of μ_{max} (Doran, 1997). The value of K_s is usually small, but K_s value obtained in this study as shown in Table 1 is quite high due to high substrate concentration which is commonly found in biofuel production (Drapcho et al., 2008).

Contois model

Contois kinetic is another unstructured model with a slight modification of Monod model:

$$\mu = \frac{\mu_{max}\, S}{K_x X + S} \tag{5}$$

Where, K_X is the Contois kinetic constant. The effective substrate concentration is proportionally related to the cell growth where μ is inversely related to the cell growth at high cell density (Dunn et al., 2003; Khavarpour et al., 2011). Therefore, Contois kinetic depicts

Table 1. Kinetic parameters of ethanol fermentation of S. cerevisiae

Parameter	Value
μ_{max}	0.084 h^{-1}
K_s	213.6 g/L
Y_{XS}	0.136 g g^{-1}
Y_{PX}	4.913 g g^{-1}
Y_{PS}	0.6682 g g^{-1}
q_p	0.4277 h^{-1}

Where, YPX is yield of product from biomass (g ethanol/g biomass) and YPS is yield of product from substrate (g ethanol/g glucose).

substrate limitation at high cell density (Billington, 1988; Vatcheva et al., 2006; Boudreau and McMillan, 2007). Diffusion limitation in flocculating or immobilized biomass is well represented by Contois model (Dunn et al., 2003).

Modified Monod model

The modified Monod model illustrates the influence of initial substrate concentration, S_0, on growth rates (Dunn et al., 2003):

$$\mu = \frac{\mu_{max} \, S}{K_s S_0 + S} \tag{6}$$

Teisser model

Teisser model expresses the growth kinetic by relating μ to S exponentially (Bailey and Ollis, 1986; Dunn et al., 2003; Khavarpour et al., 2011). It is basically an unstructured model that is adapted from Monod model given by the following equation (Mulchandani and Luong, 1989; Beyenal et al., 2003):

$$\mu = \mu_{max} \left(1 - exp^{\frac{s}{K_s}} \right) \tag{7}$$

RESULTS AND DISCUSSION

The initial cell concentration of 2.12 g/L and initial glucose concentration of 240 g/L had produced 206 g/L of ethanol after 72 h batch fermentation using *S. cerevisiae* strain in 10 L bioreactor. The kinetic parameters of this experiment are shown in Table 1.

It can be observed from Figure 1 that all the four models fit closely to the experimental data. Therefore, it is important to look for other parameters in order to determine the best fit model such as correlation coefficient and variance. Correlation coefficient (R^2) is a measure of the degree of fitness of the data to the model equation, where if $R^2 \approx 1$, the regression model is accurate (Annuar et al., 2008). R^2 which is also known as coefficient of multiple determination can be defined as follows (Montgomery et al., 2001):

$$R^2 = 1 - \frac{SS_E}{SS_T} \tag{8}$$

$$SS_E - \left[\begin{array}{c} Experimental\ value\ - \\ Calculated\ value \end{array} \right]^2 \tag{9}$$

$$SS_T = \left[\begin{array}{c} Experimental\ value - \\ Mean\ of \\ experimental\ value \end{array} \right]^2 \tag{10}$$

In addition, variance (σ^2) depicts the accuracy of experimental data to the calculated data obtained from the four models. Variance is estimated as (Montgomery et al., 2001):

$$\sigma^2 = \frac{SS_E}{n-2} \tag{11}$$

Where, n is the total number of data used in the calculation.

Correlation coefficient and variance of all four models are described in Table 2. It can be concluded from Table 2 that Teisser model marginally fit better than the rest of the models as the correlation coefficient ≈ 1. The difference in variance values between all four models shown in Table 2 is not significant. Since Teisser has the minimum variance amongst all models investigated in this study, it fits to the experimental data better than the rest of the models.

Moreover, residual plot displayed the difference between calculated and measured value of dependent variable. There are many cases with $R^2 \approx 1$, yet the model does not fit well to the experimental data (Annuar et al., 2008). Thus, it is advisable to construct residual plot for evaluating suitable fit of the models. The residual plot for all models is shown in Figure 2. The residual plot shows that all models fit the experimental data closely as evident from close distribution of points near y = 0 (that is, zero error).

Luedeking-Piret model

Kinetic expressions for ethanol production must include growth-associated and maintenance-associated (non-growth associated) production, as in the Luedeking-Piret model (Doran, 1997; Dunn et al., 2003) where $Y_{PX}\mu$ represents growth-associated term and β is the non growth-associated constant (h^{-1}):

$$q_p = Y_{px}\mu + \beta \tag{12}$$

From this equation, it had been found that $\beta = 0.303$ h^{-1} which is close to the value of q_p which is 0.4277 h^{-1}. It indicates that β is significantly larger than the growth associated term ($Y_{PX}\mu = 0.1247$ h^{-1}) which is a positive result for this simulation since ethanol production from
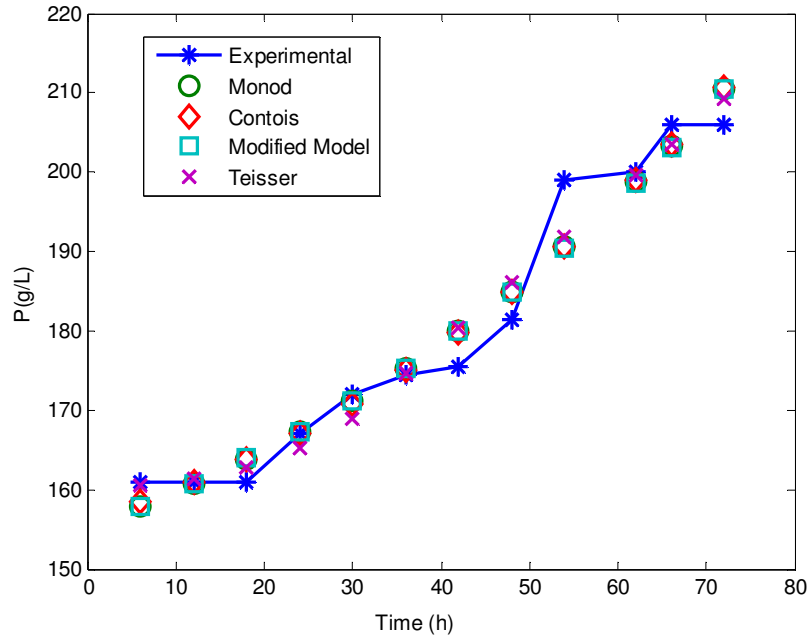
Figure 1. Ethanol concentration vs. time data from experiment and comparison with predictions from different growth kinetic models (Monod, Contois, Teisser and modified Monod models).

Table 2. The correlation coefficient and variance from data fitting of all the four models.

Model	Correlation Coefficient (R^2)	Variance (σ^2)
Monod	0.95722	16.0793
Contois	0.95849	16.8389
Modified Monod	0.95582	17.1147
Teisser	0.96299	14.3315
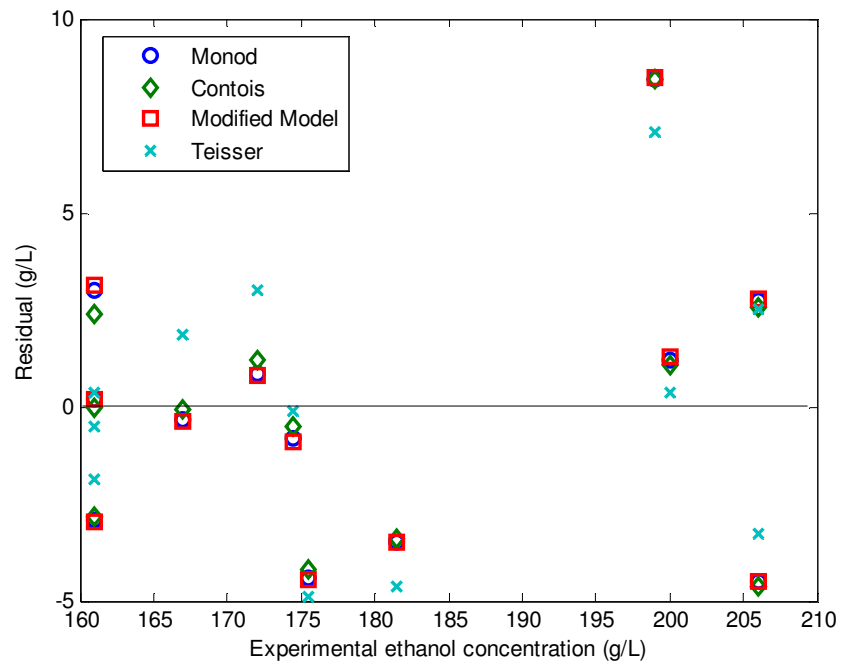
Figure 2. Residual vs. experimental ethanol concentration for different kinetic models.

yeast is indeed a non-growth associated. Ethanol production from yeast fermentation is the non-associated growth since it is excreted extracellularly by the growing yeast cells. Thus, the substrate consumed is not just for the ethanol production but also for the growth of the cells themselves. Other non-growth associated products are lactic acid and carbon dioxide.

Conclusion

Batch fermentation of *S. cerevisiae* in 10 L bioreactor produced 206 g/L of bioethanol after 72 h. The kinetic parameters of the ethanol fermentation were studied by fitting the experimental data with four different kinetic models, namely Monod, Contois, modified Monod and Teisser using MATLAB. It was found that all the four models fitted closely to the experimental data. However, Teisser model marginally fit better for ethanol production via batch fermentation of *S. cerevisiae*. Luedeking-Piret model proved that ethanol production from *S. cerevisiae* is a non-growth associated process since ethanol is the extracellular product.

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