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Kinetic Study of Mixed Glucose and Fructose Fermentation Using *Saccharomyces cerevisiae* with The Presence of Ca^{2+} Ion

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Abstract. Molasses that has high reduced polymeric sugar can be converted into fermentable sugar for ethanol production. Unfortunately, molasses also contains Ca^{2+} ion that can inhibit the hydrolysis process and can disturb the fermentation process. Therefore, this paper was conducted to study the inhibition effect of Ca^{2+} ion on fermentation process of mixed glucose and fructose using *Saccharomyces cerevisiae* through kinetic approach. The Monod model was used to study the kinetic of fermentation. The mixed glucose and fructose represented the product of molasses hydrolysis. The fermentation process was conducted at 40 g/L of reducing sugar with varying the Ca^{2+} ion content. The Ca^{2+} ion content was varying from 0 to 1.00 wt% as CaO. It has been studied that the Ca^{2+} ion can inhibit the fermentation process. The calculated yield of ethanol decreases with the increase of Ca^{2+} ion content. On the other hand, the calculated yield of biomass increases with the increase of Ca^{2+} ion content, but it decreases at a certain Ca^{2+} ion content. In order to study the fermentation kinetic, the kinetic parameters of the Monod model were obtained with or without Ca^{2+} ion content. It has been obtained that the kinetic parameters of the Monod model depended on Ca^{2+} ion content.

Keywords: Ethanol; *Saccharomyces cerevisiae*; Ca^{2+} ion; Inhibition; Kinetic; Fermentation

INTRODUCTION

The most economical sources for ethanol and citric acid fermentation is molasses which is the by-product of the sugar refinery process [1]. It has high reduced polymeric sugars content (sucrose 30-40%, glucose 5-9% and fructose 5-12%) [1,2] therefore it can be converted into fermentable sugar through enzymatic hydrolysis. The sucrose contained in molasses can be converted into glucose and fructose through enzymatic hydrolysis [3]. Then, those sugar can be converted into bioethanol through fermentation. Unfortunately, calcium ion contained in molasses could inhibit the enzymatic hydrolysis [3,4]. As known, molasses contains 0.3 – 0.9% of calcium [5].

Metal ion (calcium) in molasses could interfere the enzymatic hydrolysis, therefore it may interfere the next process of bioethanol production which is fermentation. The effect of metal ion on fermentation process has been conducted by several researchers [6–9]. Several metal ions can act as nutrition that can accelerate the growth rate or fermentation rate or can act as inhibitor that can inhibit the fermentation process.

Several researchers have studied the effect of metal ion on fermentation process. Some metal ions can accelerate the fermentation process such as K^{+}, Fe^{2+}, and Zn^{2+} [10], and some metal ions could inhibit the fermentation process such as Mg^{2+} [6], Ca^{2+} [6,10,11], Mn^{2+}, and Cu^{2+} [10]. The ion effect on fermentation process is strain based [6]. A certain metal can accelerate the fermentation using a certain biomass, but it can inhibit the other biomass. So that, there is still many challenges to study the effect of metal ions on fermentation process.

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According to Xu et al. [10] Ca^{2+} ions regulate the pH in ethanol fermentation and form structures to maintain membrane permeability. The addition of Ca^{2+} causes the work of S. cerevisiae decreases. It could decrease the fermentation efficiency of ethanol production. According to Chotineeranat et al. [11], the presence of Ca^{2+} ion could inhibit the fermentation process of ethanol production from molasses using *Saccharomyces cerevisiae*. The fermentation rate decreased with the increase of Ca^{2+} ion. The fermentation process was completely inhibited at Ca^{2+} ion of 2.16%w/v.

Many researchers just studied the effect of mineral ion on fermentation process. There is still no study about the effect of metal ion on kinetic of fermentation. Therefore, this paper studied the effect of Ca^{2+} ion as CaO on kinetic of fermentation of mixed glucose and fructose using *Saccharomyces cerevisiae*.

MATERIAL AND METHOD

Materials and Analytical Methods

Glucose and fructose were used as raw material for fermentation process. *Saccharomyces cerevisiae* was used as biomass or microbe for fermentation process. In order to determine the reducing sugar and ethanol concentration, the samples were analyzed through high-performance liquid chromatography (HPLC) [12]. The method developed by Dodić et al. [13] was used to determine the biomass concentration.

Batch Fermentation

An equal molar of glucose and fructose mixed was used as the substrate of fermentation. The fermentation was conducted at 40 g/L of substrate in a well-mixed batch reactor. Fermentation experiments were performed in a 1.2 L reactor, completed with temperature and pH control. The volume of the system was about 1 L. In order to control the pH of the fermentation medium at 4.5, 3 M of NaOH was added into the medium during the cultivation in an incubator-shaker at 37 °C and 80 rpm. Then, the dry yeast (2.5%, w/w) was added in the fermentation medium. The experiments were done for 72 h in isothermal conditions at 30 °C and monitoring with taking 6 mL samples every 6 h interval for biomass, ethanol and glucose consumption analysis. In order t study the effect of Ca^{2+} ion on fermentation process, CaO at 0.0, 0.50, 0.75, and 1.00 %wt was added to the medium.

Kinetics Model of Batch Fermentation

Kinetics model for fermentation process in a batch reactor can be described as Equation (1), (2) and (3). Equation (1) describe the biomass profile and Equation (2) describe the product concentration, then, Equation (3) describe the substrate profile.

$$\frac{dX}{dt} = \mu X \tag{1}$$

$$\frac{dP}{dt} = qX \tag{2}$$

$$\frac{dS}{dt} = -\frac{1}{Y_{x/s}}\frac{dX}{dt} - \frac{1}{Y_{p/s}}\frac{dP}{dt} \tag{3}$$

Equation (1), (2) and (3) represent the mathematical model of fermentation process where X denotes as biomass concentration, P is product (ethanol) concentration, S is substrate concentration, μ is specific growth rate, q is specific production rate, $Y_{x/s}$ is yield coefficient of biomass, and $Y_{p/s}$ represents the yield coefficient of product. The Monod model was used to describe the μ and q term as provided in Equation (4) and (5). The kinetics parameters were determined using Scilab program.

$$\mu = \frac{\mu_{max} S}{K_{sx} + S} \tag{4}$$

$$q = \frac{q_{p,max} S}{K_{sp} + S} \tag{5}$$

RESULT AND DISCUSSION

Effect of Ca^{2+} Ion on Mixed Glucose and Fructose Fermentation

The effect of Ca^{2+} ion on mixed glucose and fructose fermentation process using *Saccharomyces cerevisiae* was conducted. It is studied through the yield of product and yield of biomass obtained. As can be seen in Table 1, the dependence of Ca^{2+} ion on the yield of product and biomass was obtained.

Table 1. Ca^{2+} ions dependence on yield of product and biomass

Ca^{2+} (%wt)	Yield of Product (%)	Yield of Biomass (%)
0.00	61.67	10.55
0.50	56.76	13.37
0.75	53.76	16.41
1.00	49.66	10.64

The fermentation process was conducted with varying the Ca^{2+} ion on 40 g/L of an equal ratio of mixed glucose and fructose for 72 h. Based on Table 1, It is clear that the calculated yield of the product decreases with the increase of Ca^{2+} ion. While, the calculated yield of biomass increases with the increase of Ca^{2+} ions, but it decreases at certain Ca^{2+} ion content. The calculated yield of product decreases from 61.67% to 49.66%. On the other hand, the calculated yield of biomass increases from 10.55% to 16.41% due to the increase of Ca^{2+} ion from 0 to 0.75 wt% but it decreases to 10.64% at 1 wt% of Ca^{2+} ion content. In this case, the presence of Ca^{2+} ion interferes the fermentation process. Ca^{2+} ions can decrease the yield of the product. On the other hand, Ca^{2+} ion can increase the yield of biomass until a certain concentration of Ca^{2+} then it decreases. Walker *et al.* reported that several metal ions/minerals can affect the stability and dynamics of cell membranes of biomass during fermentation but some minerals can adversely affect yeast fermentation processes [14]. The same result was obtained. The presence of Ca^{2+} ion on fermentation can decrease the ethanol production [11]. As the increase of Ca^{2+} ion, ethanol production decreases. The decreasing of ethanol production in the presence of Ca^{2+} ion was likely caused by an inhibition effect of invertase by calcium [11] which was reported [3].

Kinetic Study of Mixed Glucose and Fructose Fermentation

Kinetic study of mixed glucose and fructose using *Saccharomyces cerevisiae* was conducted at 40 g/L of an equal ratio of mixed glucose and fructose for 72 h. The kinetic parameters are obtained and provided in Table 2. The predicted biomass concentration, ethanol (product) concentration and reducing sugar (substrate) concentration compared with data is provided in FIGURE 1.

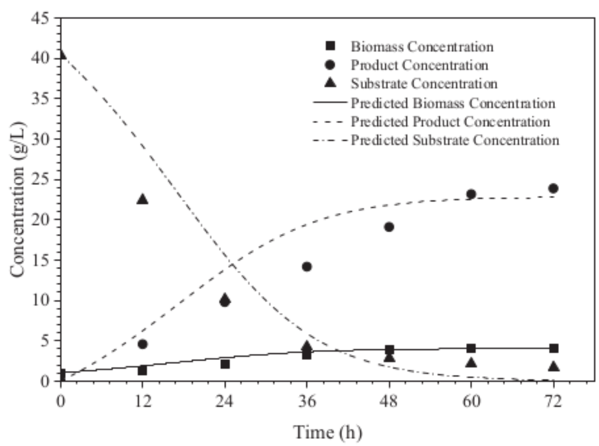

FIGURE 1. Profile comparison of data and predicted of biomass, product and substrate concentration using Monod model

Based on Table 2, the kinetic parameters of Monod model of mixed glucose and fructose fermentation using *Saccharomyces cerevisiae* are 0.183 of μ_{max}, 91.030 of K_{sx}, 1.010 of $q_{p\ max}$, 60.910 of K_{sp}, 0.150 of $Y_{x/s}$, and 1.120 of $Y_{p/s}$. As can be seen at FIGURE 1, the Monod model can describe the profile of biomass, product and substrate concentration. It has been proven with the correlation coefficient which is provided in Table 2 the correlation coefficient of Monod model for fermentation process of mixed glucose and fructose using *Saccharomyces cerevisiae* is 0.9089. Therefore, the Monod model can predict the biomass, ethanol (product) and substrate concentration as well.

The Effect of Ca^{2+} Ion on Kinetic Parameters of Fermentation

The kinetic study of mixed glucose and fructose using *Saccharomyces cerevisiae* was conducted with the presence of Ca^{2+} ion as CaO to study the effect of Ca^{2+} ion on kinetic parameters of fermentation. The Ca^{2+} ion concentration as CaO was varying from 0 – 1.00 %wt. The kinetic parameters were determined using the Monod Model which is widely used to predict the fermentation process. The kinetic parameters obtained was provided in Table 2.

Table 2. Kinetic parameters of the Monod model

Ca^{2+} (%wt)	μ_{max}	K_{sx}	$q_{p\ max}$	K_{sp}	$Y_{x/s}$	$Y_{p/s}$	R^2
0.00	0.183	91.030	1.010	60.910	0.150	1.120	0.9089
0.50	0.173	88.130	0.912	74.980	0.162	1.129	0.8755
0.75	0.166	67.988	0.998	124.510	0.198	0.985	0.8826
1.00	0.151	79.143	1.124	127.350	0.132	0.899	0.8650

As can be seen in Table 2, the value of kinetic parameters depends on the amount of Ca^{2+} ion. It can be seen that the value of maximum growth rate (μ_{max}) decreases as the increase of Ca^{2+} content. The value of the maximum growth rate decrease from 0.183 to 0.151 due to the increase of Ca^{2+} ion content. It may due to the inhibition process by Ca^{2+} ion on *Saccharomyces cerevisiae*. It has been reported that Ca^{2+} ion can decrease the fermentation efficiency by *Saccharomyces cerevisiae* to produce ethanol [10]. As can be seen, that the yield coefficient of biomass increases with the increase of Ca^{2+} ion content, but it decreases at a certain Ca^{2+} ion content. The yield coefficient of biomass increases from 0.150 to 0.198 with the presence of Ca^{2+} ion of 0 to 0.75 wt%, then it decreases to 0.132 at 1.00 wt% of Ca^{2+} ion content. On the other hand, the yield coefficient decreases with the increase of Ca^{2+} ion content. It can be concluded that the presence of Ca^{2+} ion can decrease fermentation efficiency as be reported before [10].

CONCLUSION

The kinetic study of mixed glucose and fructose fermentation using *Saccharomyces cerevisiae* was conducted with the presence of Ca^{2+} ion. It has been studied that the presence of Ca^{2+} ion can inhibit the fermentation process. It can be seen from the decrease of yield of ethanol (product) both of calculated and predicted using Monod model. The Monod model fit to the data as well as the coefficient correlation analysis. The value of maximum growth rate (μ_{max}) decreases as the increase of Ca^{2+} content. It can be concluded that the presence of Ca^{2+} ion can inhibit the fermentation process through the decrease of ethanol yield and the decrease of maximum growth rate of biomass.

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