



Research Article

## Ethanol Production from Whey by *Kluyveromyces marxianus* in Batch Fermentation System: Kinetics Parameters Estimation

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### Abstract

Whey is the liquid remaining after milk has been curdled and strained. It is a by-product of the manufacture of cheese or casein and has several commercial uses. In environmental point of view, whey is kind of waste which has high pollution level due to it's contain high organic compound with BOD and COD value 50 and 80 g/L respectively. On the other side, whey also contain an amount of lactose (4.5%-5%); lactose can be used as carbon source and raw material for producing ethanol via fermentation using yeast strain *Kluyveromyces marxianus*. The objective of this research is to investigate the kinetics of ethanol production from crude whey through fermentation using *Kluyveromyces marxianus* and to estimate the kinetics parameter using available model. The yeast was able to metabolize most of the lactose within 16 h to give 8.64 g/L ethanol, 4.43 g/L biomass, and remain the 3.122 g/L residual lactose. From the results presented it also can be concluded that common kinetic model for microbial growth, substrate consumption, and product formation is a good alternative to describe an experimental batch fermentation of *Kluyveromyces marxianus* grown on a medium composed of whey. The model was found to be capable of reflecting all batch culture phases to a certain degree of accuracy, giving the parameter value:  $\mu_{max}$ ,  $K_s$ ,  $Y_{X/S}$ ,  $\alpha$ ,  $\beta$  : 0.32, 10.52, 0.095, 1.52, and 0.11 respectively. © 2013 BCREC UNDIP. All rights reserved. (Selected Paper from International Conference on Chemical and Material Engineering (ICCME) 2012)

**Keywords:** Ethanol, Fermentation kinetics, *Kluyveromyces marxianus*, Lactose, Whey utilization

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### 1. Introduction

In order to maintaining the energy security, the development of new and renewable energy needs to be done by Indonesia stakeholder due to the population and economic growth continues to increase, as well as the depletion of oil reserves. Recently, ethanol is one of the major commodities

that were developed and used as a liquid fuel (gasoline partial substitution), and the process of making ethanol from various raw materials are widely studied. The production of ethanol in the development of first generation of renewable energy commonly used biomass containing sugar and starch crops as raw materials. The biomass

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such as sugar cane and cassava which is used in the production of ethanol, is still categorized as primary food. This development of first generation of new and renewable energy is proofed interfering the food security [1].

Since the first generation of new and renewable energy is proofed interfering the food security, the development of second generation of new and renewable energy emphasized on biomass from waste materials such as agricultural waste, and cheese industry waste (whey). Whey is an industrial waste which contained high level of pollution; to make 1 kg of cheese, 9 kg of whey is discarded to the environment. Whey contains a huge organic material with the value of BOD and COD respectively 50 and 80 g/L [2].

On the other hand, whey also contains lactose (disaccharides) about 4.5 - 5%; lactose is the carbon source which can be used as raw material for several products. Lactose can be converted into ethanol through fermentation process using yeast, especially species of *Kluyveromyces* [3]. The presence of lactose in whey as the sole carbohydrate can limit the growth of other microorganisms. This means that *Kluyveromyces* species can be optimally break down the lactose using,  $\beta$ -galactosidase enzymes. Beside lactose, whey also contains vitamins and minerals that can improve the physiological activity of cells [2]. Currently whey is utilized as animal feed, food products and alcoholic beverages [4,5]. The utilization of whey as raw material for ethanol manufacturing process cannot be implemented yet due to the development of the technology is still limited. Based on that, research on the utilization of whey as raw material for ethanol is basically needed.

Ethanol has tremendous applications in chemistry, pharmaceutical and food industries as a form of raw materials, solvents and fuel. Ethanol production worldwide in 2011 reached 23.4 billion U.S. gallons [6], where 80% of that produced by fermentation. The main aspect of this research was to study the formation of ethanol from local crude whey by *Kluyveromyces marxianus* and observe the extent of utilization of lactose in order to produce ethanol. Although many studies have been performed using de-proteined whey and yeast strain such as *Kluyveromyces marxianus*, *Kluyveromyces lactis*, *Kluyveromyces fragilis*, *Candida pseudotropicalis*, and *Saccaromyces cereviceae* [7-16], the utilization of local crude whey as a medium has not been studied in comprehensive. From economic point of view, the conversion of lactose contained in whey into ethanol is very difficult to compete with the mature technologies such as sugar cane or corn-based

ethanol. However, the presence of whey as a waste is an advantage as compared with food-based fuels such as corn. In addition, the availability of a variety of solutions for the bioremediation of whey is a valuable thing.

The kinetics of ethanol formation from local crude whey through fermentation by *Kluyveromyces marxianus* has not been intensively studied. Some researchers used a different kinds of whey and species of *Kluyveromyces marxianus* in their study [21, 22]. It has also been reported that the prediction of parameter models were based on the model development and modified from the previous kinetics model [21, 22].

This research is try to predict the parameters kinetics of ethanol production from crude whey through fermentation by *Kluyveromyces marxianus* using the common model which will gain simple and common parameters compared with previous work as it used for the same purposes. This research finding will deliver the important information for fermentor design for ethanol production from whey as lactose source and *Kluyveromyces marxianus* as microorganism. The objective of this research are to observe the kinetics of formation of ethanol from crude whey through fermentation by *Kluyveromyces marxianus*, utilize the available model for parameter prediction and to identify the extent of utilization of lactose to produce ethanol in terms of the maximum value of lactose converted into ethanol.

## **2. Experimentals**

### **2.1. Yeast Strain**

*Kluyveromyces marxianus* strain from the collection of the Center of Biomass and Renewable Energy, Chemical Engineering Department, Universitas Diponegoro (Indonesia), was the yeast strain employed in the experiments. This strain was supplied by Gadjah Mada University (Food and Nutrition Department), Indonesia. Cells of this yeast were maintained at 4 °C on potato dextrose agar (PDA) sterilized plates.

### **2.2. Culture Medium**

Cheese whey (without treatment) used as culture medium was collected from Natura Gauda cheese industry in Salatiga, Central Java, Indonesia and placed in 5 L plastic containers. The sealed containers were stored at 2-5 °C until it required in order to reduce microbial and enzymatic degradation. Whey contained 4.6% lactose and was fortified with 0.45% (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> and 0.1% yeast extract.

### 2.3. Inoculums Preparation

Inoculums was prepared by employing a loop of strain from agar slant by direct transfer in 50 ml of medium placed at 100 ml conical flask with cotton wool as a stopper. The medium was incubated at 35 °C in incubator shaker with 120 rpm agitation for 24 hour.

### 2.4. Fermentation

Batch fermentation was performed in the fermentor with 1000 ml of medium and 120 rpm agitation and temperature controlled to be 35 °C, while pH was adjusted in the initial condition to reach 4.5-5. Samples were collected every 2 hours and centrifuged at 1000 rpm for 30 minutes. The supernatant was stored at 4 °C for lactose and ethanol estimation.

### 2.5. Analytical Methods

The biomass was estimated in dry weight basis. The yeasts were harvested by centrifugation for 30 min at 1000 rpm and washed with distilled water and weighed after 2 hours at 50 °C. Lactose concentration is estimated using DNS methods [17,18] and ethanol were estimated by using dichromate colorimetric method [19].

### 2.6. Kinetic Model Prediction

The kinetic models play an important role in monitoring and predicting fermentation process of lactose. In batch fermentation the kinetic model provides information to predict the rate of cell mass and product generation. The proper cell growth (biomass) rate was described in the following form:

$$\frac{dX}{dt} = \mu X \quad (1)$$

$$\mu = \mu_{\max} \frac{S}{K_S + S} \quad (2)$$

The lactose utilization is modelled by assuming that substrate is consumed only for biomass conversion, and by combining with Monod equation, the substrate utilization can be predicted by Equation 3. While ethanol as a product of fermentation was strongly linked to biomass production. The product formation rate calculated per unit of biomass concentration are defined by the Equation 4:

$$\frac{dS}{dt} = -\mu \frac{X}{Y_{X/S}} \quad (3)$$

$$\frac{dP}{dt} = \alpha \frac{dX}{dt} + \beta X \quad (4)$$

The kinetic parameters of biomass growth were determined by *fmincon* in MATLAB using equation (1)-(4) and applying the experimental data obtained in the batch fermentation using *Kluyveromyces marxianus* and the model data in comparison. This function attempts to find a constrained minimum of a scalar function of several variables starting at an initial estimate. The parameters  $\mu_{\max}$ ,  $K_S$ ,  $Y_{X/S}$ ,  $\alpha$ ,  $\beta$  can easily be estimated on the basis of batch experiments and can rather easily be extended to describe more complex systems.

## 3. Results and Discussion

### 3.1. Growth Pattern of of *Kluyveromyces marxianus*

The batch kinetics of ethanol production from local whey was studied in detail. Figure 1 shows growth pattern of *Kluyveromyces marxianus* on whey contained 4.6% lactose. When whey as medium is inoculated with a *Kluyveromyces marxianus* strain, the organism selectively take up the dissolved nutrients from the medium and convert them into biomass and ethanol.

Growth pattern of *Kluyveromyces marxianus* (Figure 1) shows the following phase (1) lag phase, (2) exponential growth phase, and (3) deceleration phase. growth rapidly with time. Lag phase occurs immediately after inoculation and it takes 2 hours for the cells to adapt to a new environment. At the exponential phase the cells have adjusted to the new environment and the net specific growth of *Kluyveromyces marxianus* reached the maximum

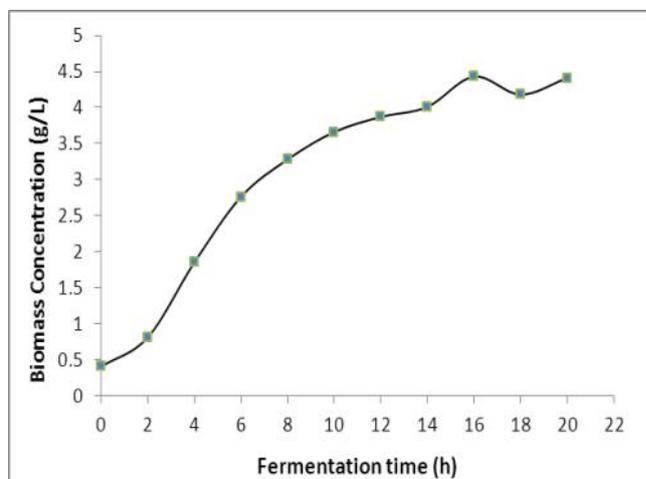


Figure 1. Growth Pattern of *Kluyveromyces marxianus* on whey contain 4.6% lactose

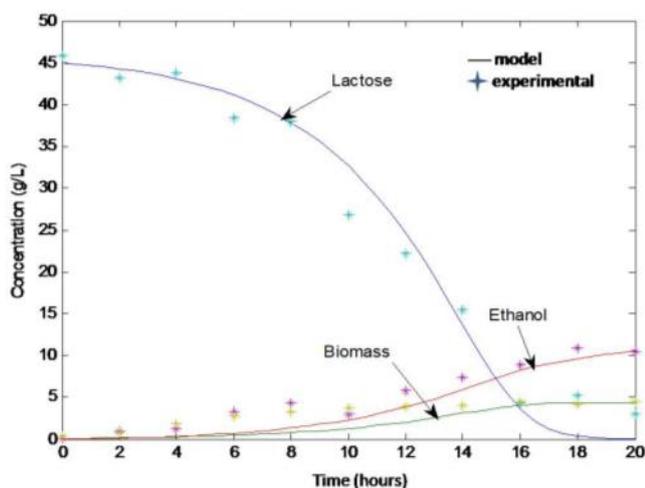
value of  $0.133 \text{ h}^{-1}$  during the exponential phase.

This result is slightly under the maximum specific growth rate recorded in other reference which were  $0.157 \text{ h}^{-1}$  [2], and  $0.15 \text{ h}^{-1}$  [20]. The main difference in between is the type of the strain and the addition micro nutrient in the medium. Although they contained all the nutrients needed and there is no need any additional supplementation [20], still growing microorganism in whey medium show an obvious uncoupling between growth and ethanol production.

### 3.2. Prediction of Kinetic Parameters

Figure 2 shows the experimental kinetics of batch culture by *Kluyveromyces marxianus* and bioconversion of whey to ethanol in temperature  $35 \text{ }^\circ\text{C}$  and initial pH 4.6. Most of the initial lactose (46 g/L) was metabolized by the yeast within 16 hours and give 8.64 g/L ethanol formation and produce 4.43 g/L biomass. While Zafar & Owais [2] claimed that the lactose mostly was metabolized by the yeast by 22 h while other [21] was 17.5 h. The difference was showed due to the difference of strain code and the additional nutrients added in the medium.

Yield product from substrate in this case ethanol from lactose ( $Y_{P/S}$ ) is  $0.213 \text{ gP/gS}$ , while yield of biomass ( $Y_{X/S}$ ) from initial lactose is  $0.097 \text{ gX/gS}$ . This is lower than the maximum theoretical yield which reached  $0.53 \text{ gP/gS}$ . This is due to the parameter of fermentation still far away from optimum and it needs further research in order to maximizing the yield of ethanol production.



**Figure 2.** Model and experimental data comparison of whey fermentation kinetics using *Kluyveromyces marxianus*

**Table 1.** Kinetic parameters in the fermentation model

Parameter	Unit	Value	Ref. 1 [21]	Ref. 1 [22]
$\mu_{\max}$	$\text{h}^{-1}$	0.32	0.55	0.401
$K_s$	$\text{g/L}^{-1}$	10.52	20	16.068
$Y_{X/S}$	$\text{gXgS}^{-1}$	0.095	0.25	0.219
$\alpha$	(-)	1.52	-	-
$\beta$	$\text{g/L h}^{-1}$	0.11	-	-

The kinetic parameters of fermentation model were determined by confirming model (Equation 1 — 4) with experimental data. The usual approach for mathematical modelling of bioreactors considers isothermal systems and is based on a single growth rate with variants of Monod kinetics. The models derived from Monod kinetics are simple in nature and easy to formulate. The prediction used a Matlab program for parameter estimation (fmincon). r as:  $\mu_{\max}$ ,  $K_s$ ,  $Y_{X/S}$ ,  $\alpha$ ,  $\beta$  with the error or fitness between the experimental and the predicted data  $R^2 = 0.785$ .

Figure 2 also shows the model and experimental data comparison of whey fermentation kinetics using *Kluyveromyces marxianus* and Table 1 shows the predicted parameters for ethanol fermentation. The predicted value for  $\mu_{\max}$ ,  $K_s$ , and  $Y_{X/S}$  was lower than the reference [21] and [22]. This differences might be due to the different species of *Kluyveromyces* employed in the fermentation process and also different operating procedures or different modelling strategies. In this work, the batch fermentation was used *Kluyveromyces marxianus* and with operating temperature  $35 \text{ }^\circ\text{C}$  and initial pH 4.6. While, the fermentation of whey by *Kluyveromyces marxianus* strain MTCC 1288 was conducted under temperature  $34 \text{ }^\circ\text{C}$  and maintained at pH 4.5 [21]. Other, *Kluyveromyces marxianus* CBS 6556 was used in whey fermentation under temperature  $30 \text{ }^\circ\text{C}$  and maintained pH 5.5 [22].

### 4. Conclusions

The yeast was able to metabolize most of the lactose within 16 h to give 8.64 g/L ethanol, 4.43 g/L biomass, and remain the 3.122 g/L residual lactose. The net specific growth of *Kluyveromyces marxianus* reached the maximum value of  $0.133 \text{ h}^{-1}$  which was slightly under the maximum specific

growth rate recorded in other reference due to the difference between the type of the strain and the addition micro nutrient in the medium. Yield product from substrate in this case ethanol from lactose ( $Y_{P/S}$ ) is 0.213 gP/gS, while yield of biomass from initial lactose is 0.097 gX/gS. From the results presented it also can be concluded that common kinetic model for microbial growth, substrate consumption, and product formation is a good alternative to describe an experimental batch fermentation of *Kluyveromyces marxianus* grown on a medium composed of whey. The model was found to be capable of reflecting all batch culture phases to a certain degree of accuracy, giving the parameter value:  $\mu_{max}$ ,  $K_s$ ,  $Y_{X/S}$ ,  $\alpha$ ,  $\beta$  : 0.32 h<sup>-1</sup>, 10.52 g/L, 0.095 gX/gS, 1.52, and 0.11 g/Lh<sup>-1</sup> respectively. This model can be used to obtain data prediction for the fermentor design activities. Further, the development of ethanol production from whey by fermentation using *Kluyveromyces marxianus* can be directed to the optimization of fermentation and the design and operating mode of the fermentors.

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#### Nomenclature

$K_s$	saturation constant (g/L)
$P$	ethanol concentration (g/L)
$S$	lactose concentration (g/L)
$X$	biomass concentration (g/L)
$t$	time (h)
$Y_{P/S}$	yield coefficient for product on substrate (kgP/kgS)
$Y_{X/S}$	yield coefficient for cells on substrate (kgX/kgS)
$\mu$	specific growth rate (h <sup>-1</sup> )
$\mu_{max}$	maximum specific growth rate (h <sup>-1</sup> )

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