

Kinetics of Ethanol Production from Whey by Fermentation using *Kluyveromyces marxianus*

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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 ethanol production kinetics from crude whey through fermentation using *Kluyveromyces marxianus* and to identify the lactose utilization in ethanol production. 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.

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

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, bioethanol is one of the major commodities that were developed and used as a liquid fuel (gasoline partial substitution), and the process of making bioethanol from various raw materials are widely studied. The production of bioethanol in the development of first generation of renewable energy commonly used biomass containing sugar and starch crops as raw materials. The biomass such as sugar cane and cassava which is used in the production of bioethanol, 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 invorenment. 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 (disacaride) about 4.5 - 5%; lactose is the carbon source which can be used as raw material for several products. Lactose can be converted into bioethanol through fermentation process using yeast (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, β -galactosidase enzymes. Beside lactose, whey also contain vitamins and minerals that can improve the physiological activity of cells [2]. Currently whey is utilize as animal feed, food products and alcoholic beverages [4, 5]. The utilization of whey as raw material for ethanol manufacturing process can not 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 bioethanol is basicaly 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 bioethanol. Although many studies have been performed using deprotenized whey and yeast strain such as *Kluyveromyces marxianus*, *Kluyveromyces lactis*, *Kluyveromyces fragilis*, *Candida pseudotropicalis*, and

Saccaromyces sereviceae [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 objective of this research are to observe the kinetics of formation of ethanol from crude whey through fermentation by *Kluyveromyces marxianus* and to identify the extent of utilization of lactose to produce ethanol in terms of the maximum value of lactose converted into ethanol.

2. Materials and Methods

2.1. Yeast Strain

Kluyveromyces marxianus strain from the collection of the Center of Biomass and Renewable Energy, Chemical Engineering, Diponegoro University (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% (NH₄)₃SO₄ and 0.1% yeast extract.

2.3. Inoculum Preparation

Inoculum 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 150 rpm agitation for 24 hour.

2.4. Fermentation

Batch fermentation was performed in the fermenter with 1000 ml of medium and 150 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].

3. Result and Discussion

The batch kinetics of ethanol production from local whey was studied in detail. Fig. 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* (Fig. 1) shows the following phase (1) lag phase, (2) exponential growth phase, and (3) deceleration phase. 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 growth rapidly with time.

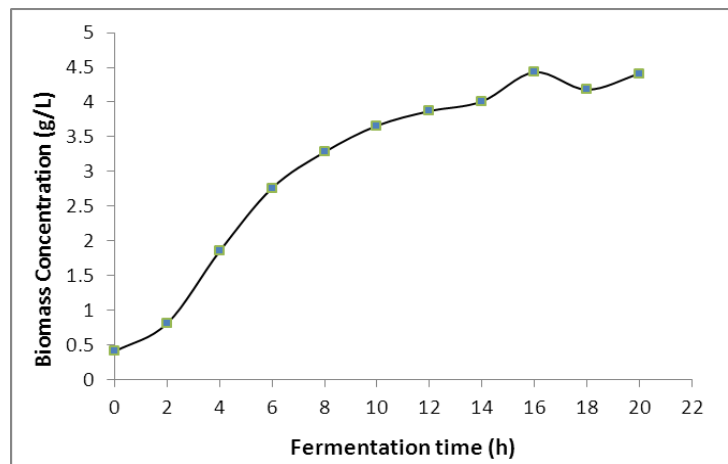


Fig 1. Growth Pattern of *Kluyveromyces marxianus* on whey contain 4.6% lactose

The net specific growth rate can be determined using below equation:

$$\frac{dX}{dt} = \mu_{\text{net}} X$$

Where X is biomass concentration, μ_{net} is the net specific growth, and t is time.

The net specific growth of *Kluyveromyces marxianus* reached the maximum value of 0.09 h^{-1} . This result is slightly under the maximum specific growth rate recorded in other reference which were 0.157 h^{-1} [2], and 0.15 h^{-1} [20]. The main difference in between is the type of the strain and the addition micro nutrient in the medium. Although whey 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.

Logistic equations are set of equations that characterize growth in terms of carrying capacity. Refer from the biomass data of *Kluyveromyces marxianus* in whey fermentation, the value of k as carrying capacity coefficient was 0.425 h^{-1} . This value was obtained from the biomass data fitted in the logistic equation as below and represented in the Table 1:

$$\frac{dX}{dt} = \mu_{\text{g}} X = k \left(1 - \frac{\bar{X}}{X_{\infty}} \right) X$$

Where X is biomass concentration, X_{∞} is the carrying capacity about 4.43 g/L since the growth almost complete at 16 h (Fig. 2).

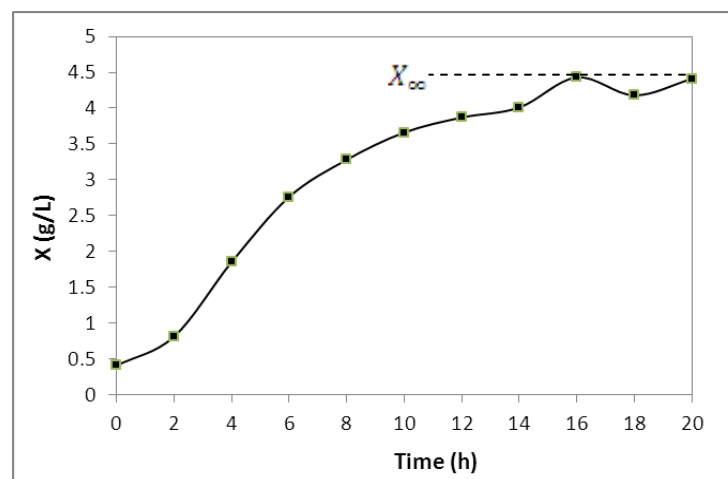


Fig 2. Logistic growth curve

Table 1.
Biomass data fitted in the logistic equation

Δt (h^{-1})	\bar{X} (g/L)	$\frac{1}{\bar{X}} \frac{\Delta X}{\Delta t}$ (h^{-1})	$\left(1 - \frac{X}{X_{\infty}}\right)$	k (h^{-1})
2	0.608	0.332	0.863	0.385
2	1.330	0.391	0.700	0.559
2	2.305	0.197	0.480	0.412
2	3.020	0.086	0.318	0.270
2	3.470	0.055	0.217	0.253
2	3.765	0.028	0.150	0.186
2	3.940	0.018	0.111	0.161
2	4.220	0.050	0.047	1.050
2	4.295	0.027	0.030	0.879

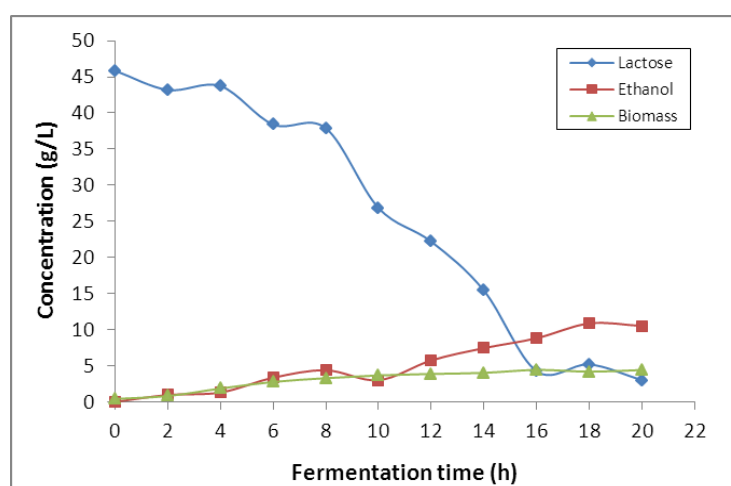


Fig 3. Experimental kinetics of batch culture of whey by *Kluyveromyces marxianus*

Figure 3 shows the experimental kinetics of batch culture by *Kluyveromyces marxianus* and bioconversion of whey to ethanol in temperature 35°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 [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 gP/gS, while yield of biomass from initial lactose is 0.097 gX/gS. This is lower than the maximum theoretical yield which reached 0.53 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.

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 0.09 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. Plotting the biomass data to the logistic equation resulting the constant k value 0.425 h^{-1} . 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. 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	carrying capacity constanta	X	biomass concentration
P	ethanol concentration	μ	specific growth rate
S	lactose concentration		

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