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Batch and Fed-Batch Fermentation System on Ethanol Production from Whey using *Kluyveromyces marxianus*

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ABSTRACT: Nowadays reserve of fossil fuel has gradually depleted. This condition forces many researchers to find energy alternatives which is renewable and sustainable in the future. Ethanol derived from cheese industrial waste (whey) using fermentation process can be a new perspective in order to secure both energy and environment. The aim of this study was to compare the operation modes (batch and fed-batch) of fermentation system on ethanol production from whey using *Kluyveromyces marxianus*. The result showed that the fermentation process for ethanol production by fed-batch system was higher at some point of parameters compared with batch system. Growth rate and ethanol yield ($Y_{P/S}$) of fed-batch fermentation were 0.122/h and 0.21 gP/gS respectively; growth rate and ethanol yield ($Y_{P/S}$) of batch fermentation were 0.107/h, and 0.12 g ethanol/g substrate, respectively. Based on the data of biomass and ethanol concentrations, the fermentation process for ethanol production by fed-batch system were higher at some point of parameters compared to batch system. Periodic substrate addition performed on fed-batch system leads the yeast growth in low substrate concentrations and consequently increasing their activity and ethanol productivity.

Keywords: batch, ethanol. fed-batch, fermentation, *Kluyveromyces marxianus*, whey

1. Introduction

Nowadays the reserve of fossil fuel has gradually depleted. This condition forces many researchers attempt to find energy alternatives which is renewable and sustainable in the future. The conversion of agricultural products into biofuels and chemicals has attracted many researchers in order to do the intensive works in renewable energy (Foda, *et al.* 2010). Recently, ethanol is considered as one of the major commodities that are developed and used as a liquid fuel (gasoline partial substitution), and furthermore the process of making ethanol from various raw materials were widely studied.

The first generation ethanol was converted from biomass containing sugar and starch crops as raw materials. The biomass such as sugar cane and cassava is still categorized as food sources (Ariyanti & Hadiyanto 2012). The utilization of agricultural products as raw materials for ethanol production has major drawback, it still competes with food security program. Based on that, it is important to find out another raw material that can be used as an alternative for ethanol production. Cheese whey derived from dairy industry waste can be used as raw material for ethanol production, since it contained 4.5-5% of lactose (Shahani, *et al.* 1980; Toyoda & Kazuhisa 2008).

The conversion of whey into ethanol is commonly conducted in fermentor using yeasts such as *Kluyveromyces lactis*, *Kluyveromyces marxianus* & *Candida pseudotropicalis*. According to Toyoda and Kazuki (2008) ethanol concentration of 8.0 g/L can be achieved by employing *Kluyveromyces lactis* and using whey as substrate. While Zafar and Owais (2005) found that ethanol concentration was achieved at 8.9 g/L by using crude whey as substrate and yeast *Kluyveromyces marxianus*.

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The simplest system of bioreactor used in fermentation process is under a batch operation. In batch system, the substrate is fed at the beginning of the process and nothing is added or taken during the fermentation process. This system may lead to the low ethanol yield and productivity, by substrate inhibition phenomena (Cheng, *et al.* 2009). Previous showed that the production of ethanol can also be performed using fed-batch system (Saarela, *et al.* 2003; Lukondeh, *et al.* 2005; Rech, *et al.* 2006; Cheng, *et al.* 2009). At fed-batch system, the substrate is periodically added during fermentation process until maximum volume is reached. Fed-batch offers many advantages compared to the batch system. Fed-batch system helps to prevent substrate inhibition during fermentation (Cheng, *et al.* 2009). Lukondeh *et al.* (2005) stated that production of ethanol in fed-batch system could acheieved 0.38 g/g of biomass concentration by employing *Kluyveromyces marxianus* and using lactose as substrate.

Comparison between batch and fed-batch fermentation in ethanol production has been carried out by Rech and Ayub (2006). They used whey powder as a substrate and *Saccharomyces cerevisiae* as a microorganism. It was reported that the yield of biomass in batch system was 0.18 [unit?]. Meanwhile in fed-batch system, the yield was 0.23 [unit?]. The objective of this research is to compare the performances of batch and fed-batch system that used in whey fermentation by *Kluyveromyces marxianus*. This research finding will deliver important information for fermentor design of ethanol production from whey as lactose source and *Kluyveromycess marxianus* as microorganism.

2. Material and Method

2.1 Microorganism

Yeast *Kluyveromyces marxianus* was obtained from Center of Biomass and Renewable Energy (C-BIORE), Chemical Engineering Department, Diponegoro University c. Cells of this yeast were maintained at 4^{o}C on potato dextrose agar (PDA) sterilized plates.

2.2 Culture Medium

Cheese whey from PT. Baros Bukit Cempaka Branch Salatiga, Central Java was stored at 2-5^{0}C. It contains 4.6% lactose and supplemented with 0.1% yeast extract.

2.3 Inoculum Preparation

One colony of microorganisms was cultured in a 100 ml erlenmeyer flask, containing 20 ml of culture medium. The flask was then incubated at 30^{0}C for 24 hours on incubator shaker (STUART) at 150 rpm.

2.4 Fermentation

Batch Fermentation

Batch fermentation was carried out in a 1000 ml erlenmeyer flask, with 500 ml of total volume of fermentation medium. About 480 ml medium was inoculated with 20 ml inoculums and acidity was adjusted to pH 4.5. The fermentation process was carried out at 150 rpm, temperature of 30^{0}C in incubator shaker (STUART) for 24 hours. Samples were taken every 2 hours for analysis.

Fed-batch Fermentation

Fed-batch fermentation was carried out in 1000 ml erlenmeyer flask, with a total volume of 500 ml. About 30 ml of medium were inoculated with 20 ml of inoculums, and initial pH was set at 4.5. The additions of culture media were conducted continuously every hour, until it achieved a total volume of 500 ml. It was carried out at 150 rpm, temperature of 30^{0}C in incubator shaker (STUART). Samples were taken every 2 hours for further analysis.

2.5 Analytical Methods

Biomass concentration was determined by measuring the optical density of the fermentation broth sample at 620nm (A_{620}) with a spectrophotometer (OPTIMA SP-300). A calibration curve was used to convert the optical density data to the dry biomass concentration. Ethanol concentration was analyzed using potassium dichromate method and lactose concentration was analyzed using DNS colorimetric method.

3. Result and Discussion

3.1 Batch Fermentation

Fermentation was carried out in a batch system, with initial whey concentration of 4.6% for 24 hours at temperature of 30^{0}C and initial pH 4.5. The growth pattern of *Kluyveromyces marxianus* in batch fermentation was depicted in Fig. 1. Exponential phase was reached at 2-4 hours, the deceleration phase takes place at 4-10 hours, and the stationary phase takes place at 10-18 hours. Lag phase was not observed which may occur between 0-2 hours. At exponential phase, the concentration of microorganisms rapidly so that the number of cells exponentially increase. After that, the deceleration phase occurs for six hours, because of the concentration substrate is reduced, and the growth rate of microorganism also decreases until finally reaching the stationary phase.

Lactose concentration decreases significantly in the first ten hours simultaneously with the increment of biomass and ethanol productions.

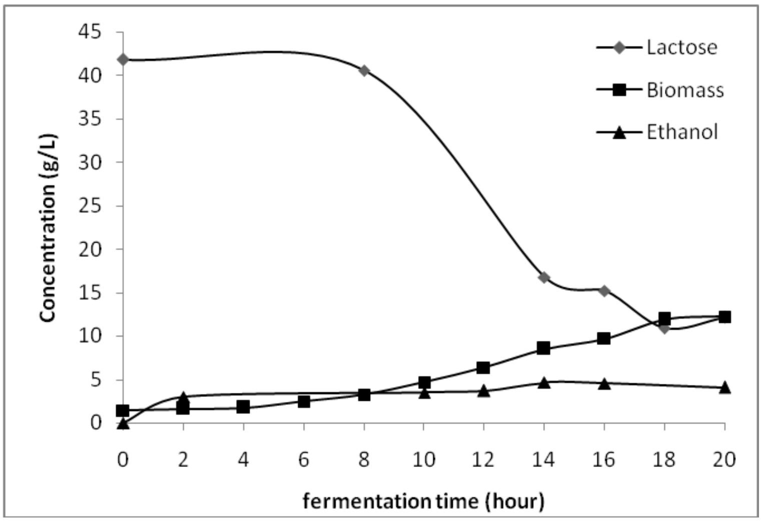


Fig. 1 Ethanol fermentation profile of cheese whey with *Kluyveromyces marxianus* in a batch process temperature 30^{0}C, and initial pH 4.5

Table 1
Parameters of batch fermentation in several researches

Parameters	This research 30^{0}C	Becerra & Siso (2006) 30^{0}C	Ariyanti & Hadiyanto (2012) 35^{0}C
μ (hour^{-1})	0,107	0,076	0,133
$Y_{p/s}$ (g/g)	0,12	-	0,213

It occurs because the cells consume the lactose for 10 hours. Growth pattern, presented in Fig. 1, shows that biomass concentration reached its maximum value of 12.245 g/L at 20 hours of fermentation for the batch run, which produced biomass yield ($Y_{X/S}$) of 0.364 g biomass/gsubstrate and a growth rate of 0.107/h (Table 1), less then results obtained by Ariyanti & Hadiyanto (2012) who reported a growth rate of 0.133/h for *Kluyveromyces marxianus* cultivated in 46 g/L of cheese whey at temperature of 35^{o}C. Higher temperatures can cause the reduction in the activity or inactivation of the enzymes *β-galactosidase,* and it shows by the results that the biomass which becomes lower (Kourkoutas 2001). However different phenomena can be detected in the use of *Kluyveromyces marxianus* as a strain to convert lactose to ethanol. This strain has thermotolerant property which can survive up till 52^{o}C (Guimarães, *et al.* 2010; Goshima, *et al.* 2013).

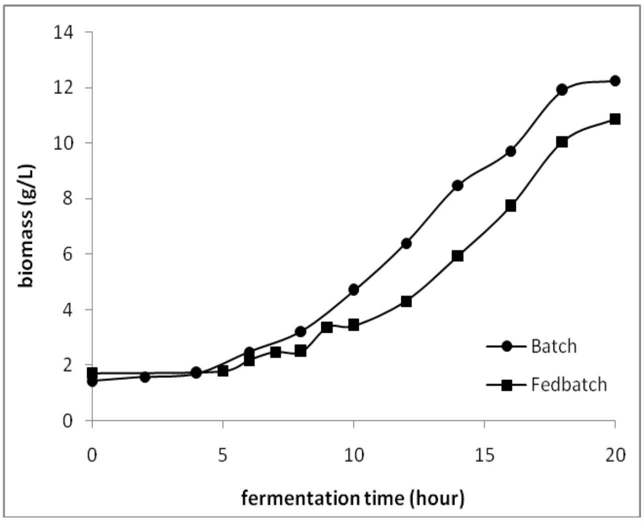
The ethanol concentrations obtained (4.6362 g/L) for 14 hours has a higher concentration than the ethanol produced during 22 hours in the research by Zafar & Owais in 2005 (2.10 g/L). Ethanol yield obtained in this research (0.12 gP/gS) was lower than research that has been done by Ariyanti & Hadiyanto (2012) (=0.213 gP/gS) by using temperature of 35^{0}C. This was probably occurred because at higher temperature the yeast *Kluyveromyces marxianus* tends to increase their activity and biomass production which lead to the higher production of ethanol. Another reason is that at lower temperature,the ethanol product might be used as a carbon source by microorganisms. So the ethanol yield becomes lower than the result of previous research.

3.2 Comparison of Fermentation Batch and Fed-batch

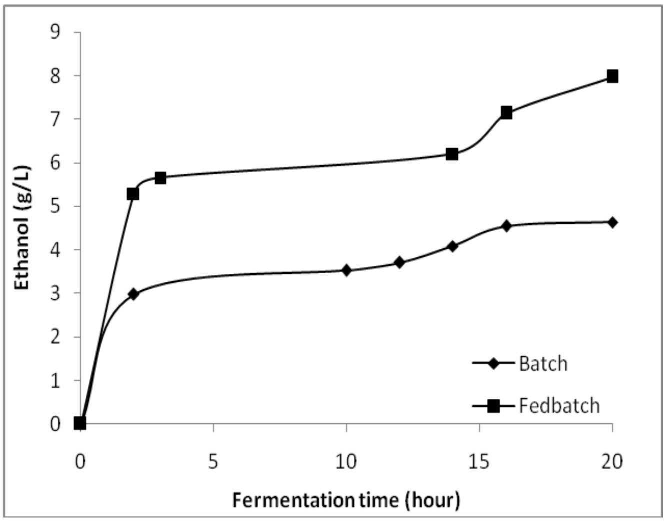
Fed-batch fermentation was performed for 20 hours with a controlled temperature of 30^{0}C and initial pH 4.5. Substrate was added continuously about 58.5 ml/h to the fed-batch fermentation system. This addition of feed were calculated based on the value of previous batch fermentation growth rate 0.107/h.

The comparison results between batch and fed-batch system of cheese whey fermentation using *Kluyveromyces marxianus* can be shown in Fig. 2. There are also comparison of biomass and ethanol production of each system. Data in Fig. 2 can be used as a based calculation for parameters of the fermentation process such growth rate, biomass productivity, and yield ethanol which presented in Table 2. Growth rate in the batch fermentation was 0.107/h, while growth rate in fed-batch fermentation was 0.122/h. It was slightly different and fed-batch system shows a better result.

Comparison of ethanol production by fermentation batch and fed-batch was shown in Fig. 2 (b). Ethanol concentrations in batch fermentation were 4.6362 g/L with ethanol yield ($Y_{P/S}$) of 0.12 gP/gS. Ethanol concentration and ethanol yield in fed-batch were 7.9626 g/L, and 0.21 gP/gS, respectively. This numbers shows that fed-batch system performed better than batch system in cheese whey fermentation using *Kluyveromyces marxianus*. This is supported by other researchers who explained that the productivity of enzymes and ethanol production in fed-batch fermentation was better than batch fermentation (Rech & Ayub 2006; Rech & Ayub 2007).



(a)



(b)

Fig. 2 Comparison of biomass (a) and ethanol production (b) between batch and fed-batch fermentation at a temperature of 30^{0}C, and initial pH 4.5

Table 2
Comparation of fermentation parameters in batch and fed-batch system

Parameters	*Batch*	*Fed-batch*
μ (h^{-1})	0,107	0,122
$Y_{p/s}$ (g/g)	0,12	0,21
Ethanol productivity (g/Lh)	1,37	4,46

Periodic substrate addition performed on fed-batch system make the yeast growth in low substrate concentrations, which lead to an increase of their activity and ethanol productivity. Yield products from substrate, in this case ethanol from lactose ($Y_{P/S}$) were 0.12 gP/gS for batch system and 0,21 gP/gS for fed-batch system. This is lower than the maximum theoretical yield which reached 0.53 gP/gS (Sansonetti 2009). This may due to the kinetic parameter of fermentation still deviate from its optimum and it needs further study in order to maximize the yield of ethanol production.

The addition of the substrate at the fed-batch fermentation process were conducted periodically. This technique can prevent substrate inhibition during fermentation (Cheng, *et al.* 2009). Moreover, the growth of *Kluyveromyces marxianus* can be controlled and the source of carbon will always sufficient to provide food needed by the yeast to grow and to produce ethanol. In line with the growth of the yeast, the production of β-galactosidase enzyme (the enzyme which responsible to transform lactose into ethanol) will also be increased and directly it will effect the ethanol productivity and production. Moreover, this method can be used to control the amount of substrate in low substrate concentration level during fermentation, so that the acceleration of product formation can be increased (Saarela, *et al.* 2003).

4. Conclusion

Based on the data of biomass and ethanol concentrations, the fermentation process for ethanol production by fed-batch system were higher at some point of parameters compared with batch system. Growth rate and ethanol yield ($Y_{P/S}$) of fed-batch fermentation were 0.122/h and 0.21 gP/gS respectively; growth rate and ethanol yield ($Y_{P/S}$) of batch fermentation were 0.107/h, and 0.12 g ethanol/g substrate, respectively. Periodic substrate addition performed on fed-batch system make the yeast growth in low substrate concentrations which is lead to increasing their activity and ethanol productivity.

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