Citric Acid Production From Molasses Use Biosynthesis Aspergillus Niger

Marlinda, Mardhiyah N, M. Irwan, Ramli

Abstract: Molasses is a by-product of the sugar processing industry. Each year, molasses are produced in large quantities. Therefore, there is necessity for further treating molasses in order to have economical values. One of the utilization efforts is by using molasses as a substrate in fermentation process of citric acid manufacture. The purpose of this study is to determine the effect of fermentation time on the production of citric acid. The variables used were fermentation time of 3, 6, 9, 12, 15, and 18 days. Molasses of 250 ml were incorporated into a fermentor with 500 ml of nutrient solution and 16 ml of Aspergillus Niger. Fermentation is carried out under aerobic conditions. The concentration of citric acid from the result of fermentation was then analyzed by using gas chromatography. From the result of analysis, the best result of citric acid was obtained at fermentation time of 9 days as much as 72.3958 g/L.

Index Terms: Aspergillus Niger, Citric Acid, Fermentation, Molasses

INTRODUCTION

Molasses is a by-product of the sugar processing industry. Molasses contain lots of sugars and organic acids [1]. Molasses content consists of 55% sucrose, 18.27% reducing sugar, 12.74% sulfuric acid, 29.25% polymer, 81.27% brick [2]. In many places this waste has very small usability and often can be problem of environmental pollution because molasses contain calcium oxide which can reduce the oxygen level of the soil. One alternative to molasses utilization is to us it as a substrate in the production process of citric acid by using microorganisms, because the molasses have high content of sugar. Citric acid is one of the most important commercial products in the world. In Indonesia, 65% of citric acid consumption is in the food and beverage industry, 20% are in the household detergent industry and the rest are in the textile, pharmaceutical, cosmetic and other industries. Citric acid can be produced either through surface fermentation culture nor submerged culture by utilizing the activity of microorganisms. Fermentation is the process of energy production in cells in anaerobic and aerobic conditions. Fermentation has an understanding of the application of microbial metabolism to convert raw materials into high value products, such as organic acids, single cell proteins, antibiotics, and biopolymers. Fermentation can be interpreted as a gradual change by enzymes of some bacteria, yeasts and fungi. Aspergillus Terreus is a fungus of the Ascomycota phyla. Its morphological features are same as the most of the species in this phylum that are fililament, hyphae secured or have septa, and can be found abundantly in nature. Aspergillus Niger can grow optimum at 35-37 °C, with a minimum temperature of 6-8 °C, and a maximum temperature of 45-47^{o}C.

In addition, in the process of growth of this fungi is as aerobic microorganisms. Aspergillus Terreus also produces several enzymes, proteases, amylases and α - glactosidases that can be used as biocatalysts in the production of citric acid by fermentation [3]. Citric acid is the primary metabolite product formed from TCA Cycle (Tricarboxylic Acid Cycle). Citric acid is the primary metabolite product formed from TCA Cycle (Tricarboxylic Acid Cycle) . Glucose is the main source of carbon in its production. In most microbes, 80% glucose is broken down through reactions in the Embden Meyerhof Parnas (EMP) pathway. The pyruvic acid which is the final product of the EMP pathway will be further oxidized and then with the assist of the decarboxylase enzyme to form acetate (decarboxylation). The acetate formed binds to the A-coenzyme produces Acetyl - CoA. Furthermore Acetyl - CoA and oxaloacetate which is one of the compounds between TCA cycle condenses to form Citric Acid with the assist of enzyme citrate oxidation synthase [4, 5].

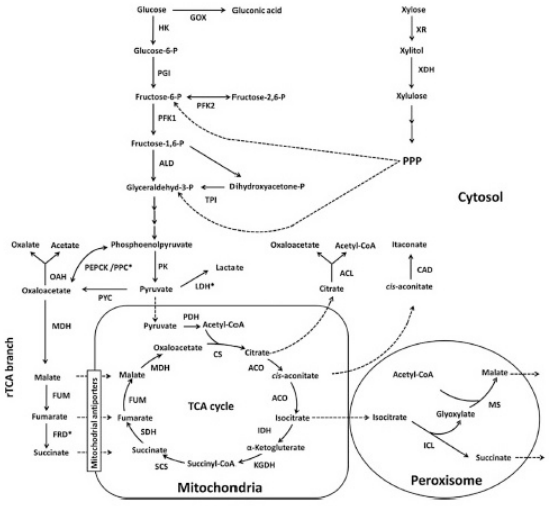

Figure 1. *Tricarboxylic Acid Cycle (Krebs Cycle)*

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2 MATERIAL AND METHODE

Materials and Tools
Materials used is molasses (20% v/v), KH2PO4, (NH4)2SO4, MgSO4.7H2O, MgO, Ca(OH)2, CuNO3.7H2O and Aspergillus Terreus. Tools used aerobic fermenter, incubator, oven, autoclave, desiccator, rotary evaporator, gas chromatography.

Breeding of Aspergillus Terreus in Liquid Media
Weighing the nutrient broth as much as 52g then insert into a beaker containing 1 L of aquadest. Stirred then inserted into autoclave, after removal from autoclave add Aspergillus Niger 2 ose. Incubate for 24 hours.

Citric Acid Production
Put molasses into an aerobic fermentor, add 500 ml of nutrtents containing KH2PO4 (NH4)2SO4, MgSO4.7H2O, MgO, Ca(OH)2, CuNO3.7H2O. Put in 16 ml of bred Aspergillus Niger. Measure and set the pH to 6 each day. After the fermentation is complete, the fermentation result is incubated for 24 hours.

Determination of Citric Acid
The amount of Citric Acid is determined by using chromatography method. Gas chromatography used is Variant 450. The injector temperature is set at 200 °C, the oven temperature is 170 °C and the detector temperature is 200 °C.

3 RESULT AND DISCUSSION

3.1. Biomass Content
This study aims to determine the effect of fermentation time on the production of citric acid from the molasses substrate by using Aspergillus Niger, so that the optimum time in the formation of citric acid is obtained. The first parameter of the analysis is the measurement of biomass. Measurement of biomass aims to show Aspergillus Niger, activity in the formation of citric acid. dead cells. In the death phase, Aspergillus Niger cells undergo lysis thus reducing the measured biomass weight. This is because the mass of cells that have been partially lost will be converted into energy utilized by living cells as an energy source for its growth.

3.2 Glucose Content
In the process of fermentation, the source of sugar used comes from the content of molasses substrate [5]. the measurement of residual sugar during fermentation aims to determine the presence of substrate on fermentation. Glucose is a source of nutrients for Aspergillus Niger as a carbon source [6]. The growth of fungi on the fermentation media is influenced by the nutrients present in the substrate and given to the substrate [7]. Decreased glucose content indicate that the source of sugar is used by microorganisms to multiply cells and survive.

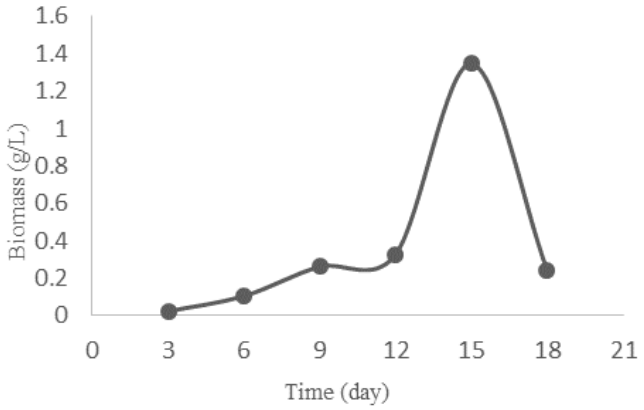

Figure 2. *Relation of time to Biomass Content*

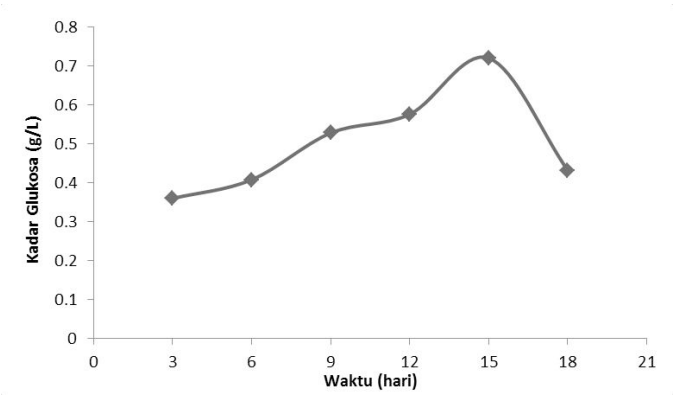

Figure 3. *Relation of time to Glukose Content*

From Figure 2 it can be seen that the content of biomass on the 3rd day increase until the 12th day, it shows that Aspergillus Niger has growth (lag phase) that fungus begin to divide itself with low speed. On the 12th day until the 15th day, the biomass content increased drastically with the concentration of biomass as much as 1,3455 g/L, this shows that the fungus is in the log phase so that the fungus divides rapidly. The speed of growth is influenced by pH, nutrient content, temperature and humidity. On the 18th day the content of biomass has decreased to 0.2422 g/L this is caused by Aspergillus Niger has entered the phase of death. Most of the microbes begin to die because the nutrients in the medium are gone [4]. Measurements of dry weight biomass not only measure the live cell only, but also In Figure 3. there is a relation of fermentation time to glucose content. On the 3rd day until the 15th day the glucose content obtained increased, ranging between 0,3600 g/L - 0,7200 g/L. The increase in glucose content occurs due to the sugar content analyzed from the molasses substrate that having high sucrose and reducing sugars high enough to convert to simpler sugars. So that sucrose composed in long carbon chains is more dominant in the substrate. Then during the fermentation time sucrose will be degraded into shorter carbon chains, glucose and fructose. But on the 18th day glucose levels decreased by 0,4320 g/L. This is because the longer the fermentation time, the less nutrient content and the source of sugar in the media, so the glucose is decreased. the remaining glucose concentration will decrease with the formation of metabolites in the form of the glycosidase enzyme

produced in the fermentation process of Aspergillus Niger in the formation of citric acid [8]. the effect of residual glucose on fermentation time decreases because Aspergillus Nige microorganisms use as macronutrients for growth so that the number of Aspergillus Niger cells increases, so that the metabolic process of Aspergillus Niger cells results in increasing cell reaction rates [9].

3.3 Citric Acid Concentration

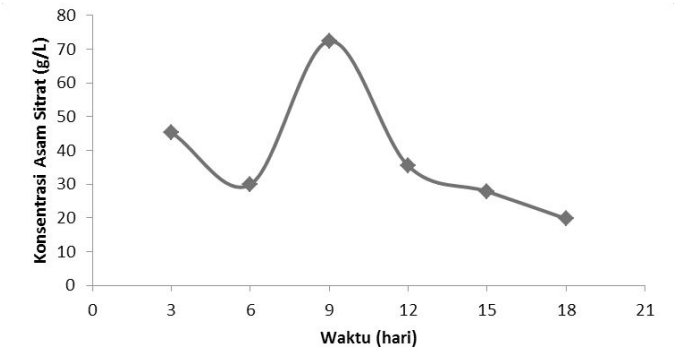

Figure 4. *Relation of time to citric acid concentration*

In Figure 4. it can be seen the relationship between fermentation time to citric acid concentration. On the 3rd day to the 6th day the concentration of citric acid obtained from the fermentation is decreased, with concentration amount of 45.3125 g/L to 29,9479 g/L. This is because the substrates used are also contained molasses of nitrogen minerals which are limiting factors The excessive availability of nitrogen minerals resulted in the metabolism of Aspergillus Niger concentrating on cell formation rather than the formation of primary metabolites, namely citric acid. On the 9th day the concentration of citric acid increased again, with the highest citric acid concentration of 72,3958 g/L. Due to the re-formation of citric acid in the TCA cycle. In one of TCA cycle, α -ketoglutarate is converted to oxaloacetate. The condensed oxaloacetate with Acetyl-CoA produces citric acid, so the TCA cycle can be re-established [11]. And entering the 12th day until the 18th day, the resulting citric acid concentration decreased by 35,4167 g/L to 19,7917 g/L. This is due to the formation of other organic acids that exist in Tricarboxylic Acid cycle path (TCA).

3.4 Amount Citric Acid

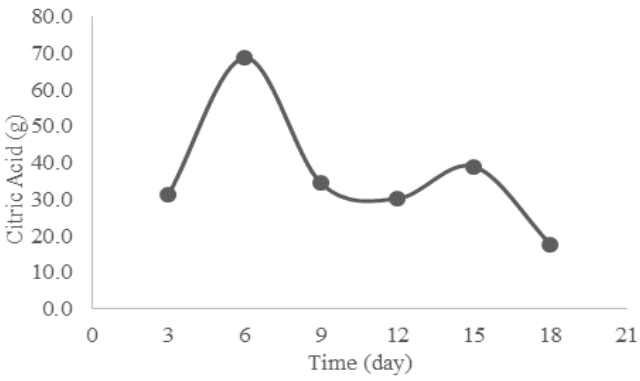

Figure 5. *Relation of time to citric acid production*

In Figure 5 we can see the relationship between fermentation time and the amount of Citric Acid. On the 3rd to 6th day the number of citric acid increases and then decreases until day 12th. Day 6th has the highest amount of citric acid which is amount of 68,7 g and on the 9th day has decreased the amount of citric acid up to day 12th, with citric acid amount of 30,2 g. This is caused by the formation of other organic acids that exist on the path cycle Tricarboxylic acid (TCA). However on the 15th day the number of citric acid increased again, with citric acid amount of 39 g. This may be due to the re-establishment of Citric Acid on the TCA Cycle. In one of TCA Cycle, αKetoglutarate is converted to Oxaloacetate [12]. The condensated oxaloacetate with Asetyl CoA produces citric acid, so Krebs Cycle can be re-established. Then, on the 18th day the amount of Citric Acid decreased. This is due to Aspergillus Niger has entered the phase of death as seen in Figure 1 where the biomass content decreased from day 15th to day 18th.

4. CONCLUSION

The longer the fermentation time will increase the number of Aspergillus Niger cells, the smaller the remaining glucose concentration and the concentration of citric acid increase. The optimum time of the fermentation of the molasses substrate using Aspergillus Niger that obtained was 6 days with the amount of Citric Acid 68,7 g and concentration citrate acid 72,3958 g/L.

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