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## Fermentation Juice Sweet Sorghum Genotip 4-183A using Batch System by Optimizing the Concentration of Inoculum and Substrate

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**Abstract.** The potential of sweet sorghum in Indonesia as a raw material for bioethanol production is very large. Sweet sorghum is a plant not a food crop. Sweet sorghum juice is a liquid containing sugar can be produced by extracting or pressing sorghum stems. The sugar content of sweet sorghum juice varies depending on the type, one of which is genotype 4-183 A. The purpose of this research is to optimize the conditions of inoculum and substrate using a batch system. The parameters to be analyzed are the percentage of bioethanol yield, total biomass or yeast cells and total sugar content. This research shows that the process of fermentation-batch of juice sweet sorghum of genotype 4-183A with 9% optimum inoculum concentration and 16% optimum glucose concentration can produce high concentrations of biethanol.

### 1. Introduction

The insistent on current energy needs is still related to the undeniable fossil-based energy and environmental issues. Most of the energy is dependent on fossil-based energy such as petroleum. Ethanol or alcohol is a renewable and clear fuel, ethanol can be obtained from fermented plant material called bioethanol. Bioethanol is an oxygenated fuel that contains 35% of oxygen that can reduce particulate matter and NOx emissions from combustion outcomes [1]-[2]. In recent years sweet sorghum is widely used as a raw material of glucose-free fermentation which potentially produces fuel oil, food, feed, and various other products [3]-[4]. Sweet sorghum is one of the most efficient plants to convert CO<sub>2</sub> into glucose compared to sugarcane and corn, so this plant promises to be a source of bioenergy, food, and livestock feed. The use of sweet sorghum rods equals sugarcane, because it contains a high concentration of glucose, which can produce ethanol through fermentation [5]-[4].

Sorghum is a potential plant to be cultivated and developed in Indonesia, especially in dry areas. Its advantages lie in the nature of the resilience to drought, high production and cost of production is relatively inexpensive, more resistant to pest and disease attack compared to other crops. Sorghum has great potential to be cultivated and developed commercially, as it has wide adaptation power, high



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productivity, relatively little input, resistance to plant pests and diseases, drought tolerant, salinity, and L [6]-[7]. Another advantage of the sorghum stems containing sweet nira with brix glucose concentration ranges between 11,00-15,96 [7].

Fermentation to produce bioethanol is the process of converting glucose by utilizing microbial performance to produce bioethanol. In batch fermentation, raw materials are added to the fermentation bottles along with microorganisms, nutrients, and other ingredients at the beginning of the whole batch fermentation followed by the recovery of ethanol. [8]-[9].

Involvement of microorganisms in glucose fermentation is an important part of bioethanol production. The number of inoculum used is one of the most important factors affecting industrial fermentation, as well as the duration of lag phases, specific growth rate, biomass yield, and final product quality [10]. *Saccharomyces cerevisiae* is a yeast strain that is often used to ferment bioethanol because it has certain characteristics such as high selectivity, high ethanol yield, high fermentation rate and low side product accumulation [11]. *S. cerevisiae* is a fairly high alcohol tolerant yeast (12-18% v/v) on a suitable medium, resistant to high glucose concentrations and remains active in fermentation at a temperature of 4-32°C [12].

To increase the productivity of bioethanol made from sweet sorghum juice, it needs to be done through the optimization of inoculum concentrations *Saccharomyces cerevisiae* and substrate to increase the yield of bioethanol. Culture preparations *S. cerevisiae* is an inoculum preparation to multiply the number of yeast as well as exercise yeast resistance [13], wherein *S. cerevisiae* of yeast used will be tested for its ability through the fermentation process in producing bioethanol Highest. Therefore, one of the efforts to produce bioethanol concentrates higher is the optimization of the inthe concentration *Saccharomyces cerevisiae* and substrate. This paper will discuss the fermentation joice sorghum sweet genotype 4-183A using a batch system, by learning the optimization of incondition conditions and optimization of substrate concentrations that produce maximum bioethanol. The parameters to be analysts are the total biomass or the Khamir cells, the total concentration of glucose and the concentration of bioethanol.

## 2. Methods

This research was carried out using the experimental methods, which include 3 stages: (1) the preparation of sweet sorghum stem of genotyp 4-183A; (2) The process of taking the juice Sorgum-sweet; (3) fermentation of juice sorghum-sweet.

### Sweet sorghum rod preparation of genotype 4-183A

The first treatment done is the preparation of raw materials done by taking the sweet sorghum stem from the harvest. Harvest is done in residential garden employees PT. Semen Tonasa Pangkep. The sorghum stems are cleaned from the leaves and dirt, weighed first to figure out the weight of the sorghum stem..

### **The process of retrieval the sweet sorghum juice**

The treatment done to acquire juice of sweet sorghum is by way of extraction. The new sweet-sorghum stem harvest is instantly taken. Stem dismembered then crushed using a blender, soak with warm water for 10 minutes. The next process is separated by filtering to obtain filtrate (juice). The resulting Juice analyzed total glucose using the method luff Schoorl [14].

### **Batch fermentation process of sweet sorghum juice**

The research is done for the optimization of inoculum concentration and substrate concentration (sweet juice sorghum). It is expected to produce the highest bioethanol concentrations of batch fermentation results. The implementation of this stage consists of 2 (two) activities, namely the inoculum *Saccharomyces cerevisiae* preparation, and the fermentation process.

#### **A. Preparations inoculum *S. cerevisiae***

The media used to grow the yeast *S. cerevisiae* is substrat, glucose, N source and trace elements. At first the substrate is inserted into the Erlenmeyer. Erlenmeyer is closed by using cotton and aluminium for further inclusion in autoclaving and sterilized at 121°C for 15 minutes. After sterilisation is complete, the erlenmeyer is removed from the autoclaves to be cooled at room temperature. The making of the starter is done by inserting the bread yeast Fermipan into the liquid media that has been sterilized. Then each erlenmeyer is closed back and fermented in incubator shake for  $\leq 48$  hours at room temperature before use in fermentation process.

#### **B. Batch Fermentation**

Fermentation is carried out by taking a substrate (sorghum-sweet juice) with a substrate concentric of 12%, 14%, 16% and 18% (b/V) and each of them is inserted into the fermentor bottle. Furthermore, the bottle is closed using cotton and aluminium foil to be inserted into the autoclaving and sterilized at 80°C for 20 minutes. After a cold solution add an Inoculum to each fermentor and close tightly. Inoculum variation uses concentrations of 8%, 9% and 10% (V/V) of the volume of substrates in bioreactors. The fermentation process is done for 72 hours. Sampling is performed for analysis with a time interval of 6 hours. Observations include total glucose concentrations, total microbes and concentrations of ethanol produced. Analysis of bioethanol concentrations using Abbe refractometers, the total determination of the calculated microbes is the number of dry cells contained in the fermentation fluid, for the concentration of glucose using the Luff Scrool method [14].

### **3. Results And Discussion**

#### **Determination of the concentration of culture *Saccharomyces cerevisiae* optimum**

The batch fermentation of substrate from the sweet juice sorghum of genotype 4-183A, with glucose concentration in total 18%. The growth curve of *Saccharomyces cerevisiae* during the fermentation process with inoculum concentrations depicted in the Figure 1. The growth of variation inoculum concentration displays the same pattern. The number of initial cell biomass for inoculum concentrations is 9% higher than other concentrations. Cell biomass reaches a maximum of 18 hours of incubation. According to Yi-H. Chang (2018) Yeast cell biomass increased to a maximum of 1.7 g/L and the pH decreased rapidly from 5.5 to 3.5 in the first 18 hours of fermentation [15]. The growing phase of *S. Cerevisiae* is divided into 3 phases, i.e. the logarithmic (exponential) phase, occurring during the first 18 hours of incubation, the slow phase occurs at the 18th hour to the 24th hour, then the stationary phase occurs at the 24th hour to the hour to-72.

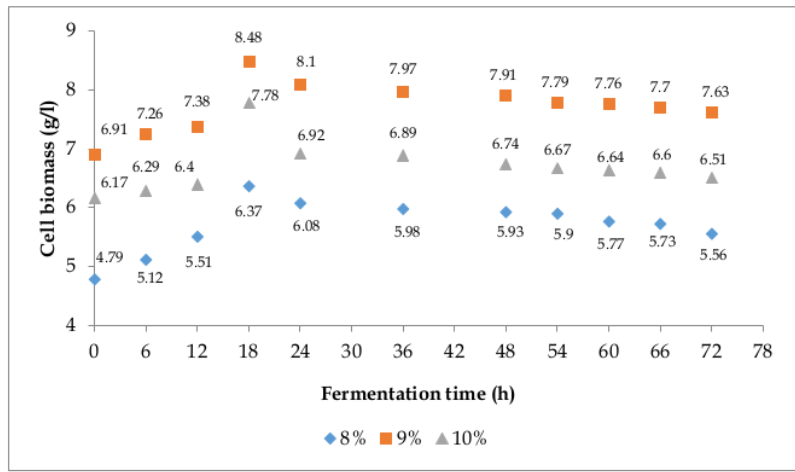


Figure 1: Growth chart of *Saccharomyces cerevisiae* with concentrations of 8%, 9% and 10% (v/v) with on fermentation time.

On the curve of Figure 1., the lag phase (adaptation phase) is not observed, because the activation has been done in the substrate medium for 48 hours, so that *S. Cerevisiae* has been adapted in medium and actively synthesizes enzymes for growth. In the lag phase, *Saccharomyces* experienced a period of adaptation with the environment and there has been no growth [16]. Yeast cells grow in three main phases namely the lag phase, the exponential phase and the silent phase. The lag phase refers to the initial growth phase, when the number of cells remains relatively constant before rapid growth, also referred to as adaptation time [16]. In the exponential phase there is an increased amount of cell biomass very rapidly. The exponential phase is the second growth phase, where cells grow most rapidly [17]. After the exponential phase, enters in the slow phase, in this phase the cell division is reduced, because the substrate concentration decreases so that the growth rate of cells decreases until it enters the stationary phase.

**Batch fermentation for optimum substrate concentration determination**

The concentration of substrates tested on this research is 12%, 14%, 16% and 18%, and the inoculum concentration used is 9%. The results of the research present that the highest cell biomass amount at 16% substrate concentration, compared to other substrate concentrations. This is due to this concentration of *S. cerevisiae* active self-splitting so that the number is more than the other substrate concentrations. Figure 2 displays the bioethanol curve, glucose concentration and total biomass during the fermentation process carried out at various concentrations of substrates. From the image seen during the fermentation process, the substrate concentration curve decreases while the cell biomass and bioethanol concentrations are increased. Glucose concentration decreases simultaneously with the increase in cell biomass and the concentration of ethanol [15].

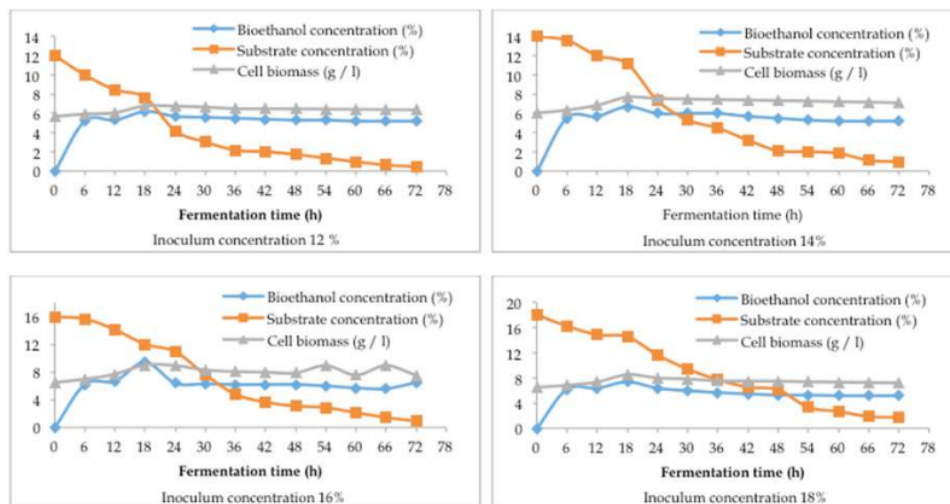


Figure 2. Graph of a batch fermentation with glucose concentrations of 12%, 14%, 16%, and 18%.

Glucose consumption to close to zero within 72 hours incubation. The concentration of ethanol produced at maximum conditions varies between 6.20% (V/V) to 9.40% (V/V). The lowest concentration of ethanol obtained from the treatment using a substrate of 12% (b/V) while the highest ethanol concentration on the treatment using a substrate with 16% glucose concentration (b/V) obtained at 18 hours incubation, and Glucose concentrations are still high. At 18% glucose concentration (b/V) also produced a lower concentration of ethanol than the treatment using a 16% substrate (b/V). This suggests that the optimum glucose concentration for fermented ethanol from the sweet sorghum juice is 16% (b/V), whereas if the glucose concentration is above and below the optimum glucose concentration, the ethanol produced is lower. This is due to the higher concentrations of sugar then the more substrate (carbon source) that can be consumed by *S. Cerevisiae*. However, the use of excessive substrate concentrations will be a barrier to the growth and formation of products by yeast [18].

#### 4. Conclusion

This research suggests that the process of fermentation batch of juice sorghum sweet genotype 4-183A with an iningestion concentration of 9% and a glucose concentration of 16% can produce high concentrations of biethanol.

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