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THE UTILIZATION OF FRUIT WASTE EXTRACT IN PASAR INDUK OSOWILANGUN SURABAYA (PIOS) INDONESIA AS A RAW MATERIAL OF ETHANOL BIOCONVERSION BY *SACCHAROMYCES CEREVISIAE*

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ABSTRACT

This study was aimed to determine the effect of the variations of extract concentration, the variation of the length of fermentation time, and the combination of both on ethanol produced. Ethanol fermentation used Pasar Induk Osowilangun Surabaya (PIOS) fruit waste's substrates that was extracted then added with 10% *Saccharomyces cerevisiae*. This study used 4x4 factorial design, 2 factors, and 3 replications. The first factor was the concentration of fruit waste's extracts (25, 50, 75 and 100%). Second factor was the length of fermentation time (3rd, 6th, 9th, and 12th days). Reducing sugar measurement used Luff Method and ethanol amount measurement used Pycnometri Method. Etanol amount was subjected to variance analysis with Two Ways Anova test (á 5%), continued with Duncan test. The treatment of fruit waste's extract concentration effected ethanol's amount, the maximum concentration in 100 % fruit waste's extract (6.9%). The treatment of fermentation time effected ethanol's amount, the optimum of fermentation time on the 9th days (5.15%). The combination treatment between the variations of fruit waste's extract concentration and fermentation time effected ethanol's amount, the maximum 100% fruit waste's extract within the optimum 9th days fermentation (8.12%).

KEY WORDS : Bioconversion, Ethanol, Fermentation, Fruit Waste's Extract, *Saccharomyces cerevisiae*

INTRODUCTION

Pasar Induk Osowilangun Surabaya (PIOS) was the center market of the largest fruit and vegetable in Surabaya. PIOS generated solid waste 46325 m^3/ day. The compositions of PIOS waste consist of 98.27% organic waste, such leftover vegetables and fruits (Rahman, 2013). The main content of fruit waste was carbohydrates. Bioconversion of fruit waste into ethanol is one of the promising technologies required (Nugraha, 2008).

Making ethanol can be used as a substitute for fossil fuels (Suryaningsih and Irhas, 2014). Energy demand in Indonesia recorded substantial growth in the period 1990-2005, which average of consumption was 4.08% per year. Reference Prawiroadmodjo and Armando (2005) also had also opined that the fuel crisis because of the high demand for energy increases. Reserves of petroleum, natural gas, and coal as long as the main energy source of fossil fuels decrease because of the energy needed was increasing with population growth.

Ethanol which made from biomass that had component sugar, starch, and cellulose is called bioethanol. Bioethanol was produced by using biochemical technologies through a process of fermentation of sugars from carbohydrate raw materials using microorganisms, such as *Saccharomyces cerevisiae* (Prihandana *et al*., 2007). Bioethanol has a low toxicity and did not cause pollution, and the combustion products was

environmentally friendly, so it's used as an alternative energy was good for review and consideration.

The ethanol research used PIOS fruit waste's extractas a raw material with fermentation proses using *Saccharomyces cerevisiae* 10% in batch culture reactors. This study was aimed to determine the effect of the variations of extract concentration, the variation of the length of fermentation time, and the combination of both on ethanol produced. Ethanol had a comprehensive benefit in a variety of industries. Therefore, it is necessary to do research about the Utilization of Fruit Waste Extract in Pasar Induk Osowilangun Surabaya (PIOS) as a Raw material of Ethanol Bioconversion by *Saccharomyces cerevisiae*.

MATERIALS AND METHODS

The process of making PIOS fruit waste's extract

PIOS fruit wastes was sorted and weighed to determine the percentage of the composition fruit wastes. Fruit waste's washed and cleaned, then juiced with juicer. Extracts that have been separately with fruit pulp, then varied by the addition of water, 25%, 50%, 75% and 100%.

Preparation of starter

Starter of *Saccharomyces cerevisiae* was made by inoculate the pure culture on Potato Dextrose Agar (PDA) to 100 ml of yeast extract liquid medium and glucose 1%. Then culture homogenized using a shaker for 4 hours and kept in an incubator to stabilize the growth of inoculum. After that, the cultures of *Saccharomyces cerevisiae* were incubated at room temperature for three days. Starter 100 ml propagated to two times 500 ml by inserting 10 ml the beginning culture in 490 ml of yeast extract liquid media and glucose 1% for every 500 ml. The quantity of *Saccharomyces cerevisiae* in the starter was calculated for the value of Optical Density (OD) and Total Plate Count (TPC) before it was put in the media fermentasi.

Fermentation ethanol

Waste's extract entered in a batch reactor then sterilized to avoid spoilage bacteria. In this study, we used 48 fermentor. The fermentation of ethanol was comparing the concentration of fruit waste's extract with water, 25%, 50%, 75%, and 100% was the first factor. Then in fermentors containing the sterile extracts was added 10% *Saccharomyces cerevisiae* for fermentation by variation the length of fermentation time, 3rd, 6th, 9th, and 12th days, was the second factor.

Distillation

The extracts filtered from *Saccharomyces cerevisiae* biomass as much as 100 ml was distilled by distillation. The distillation process at temperature 80^{o} C, then we analyzed the results of distillate ethanol

Reducing sugar measurement

Reducing sugar measurement using Luff methods.
Measurement of ethanol amount
Measurement of ethanol amount using Pycnometri Method.

Data analysis

Data were analyzed used variance analysis with Two Ways Anova test (á 5%), continued with Duncan test to determine the effect of the variations extract concentrations, the variation of the length of fermentation time, and the combination of both on ethanol produced.

RESULTS

The value of Optical Density (OD) and Total Plate Count (TPC) *Saccharomyces cerevisiae* in this study was 1.5 and 1.1 x 10^{16} CFU / ml. Based on the value of TPC, the conditions of *Saccharomyces cerevisiae* starter has been fulfilled which was at least 10^{6}.

Effect of The Variations ofPIOS Fruit Waste's Extract Concentration to Ethanol's Amount with *Saccharomyces cerevisiae*

The composition of substrate that used in this study are shown in Figure 1, watermelon waste's was the most dominant component, 4.5 kg. In Figure 2, it was shown that the more concentrated extract concentration of fruit wastes, then the ethanol

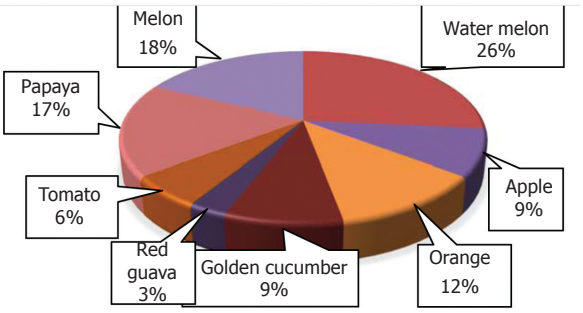


Fig. 1. Composition of PIOS fruit waste's extract that used as the ethanol substrate

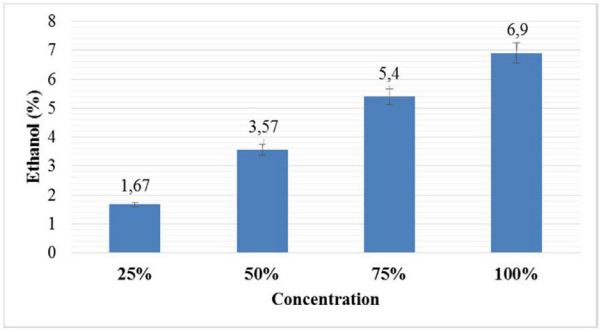


Fig. 2. The average of ethanol amount (%) to variation of the concentration of PIOS fruit waste's extract (%)

amounts produced was increased. At 100% fruit waste's extract concentration produced an average 6.9% ethanol amounts, which quite different from the concentration of 25%, 50%, and 75% of fruit waste's extract.

pH

Figure 3, in this study the pH value were in the range of 4.11 to 4.59 which in accordance with the conditions of pH optimum of *Saccharomyces cerevisiae*, 4 to 5.

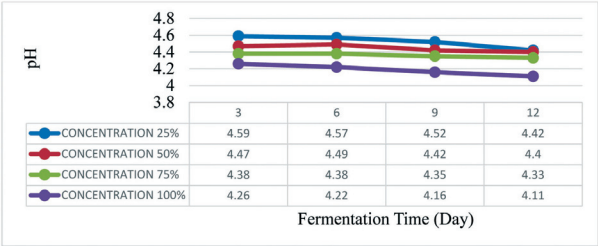


Fig. 3. Average pH value during fermentation

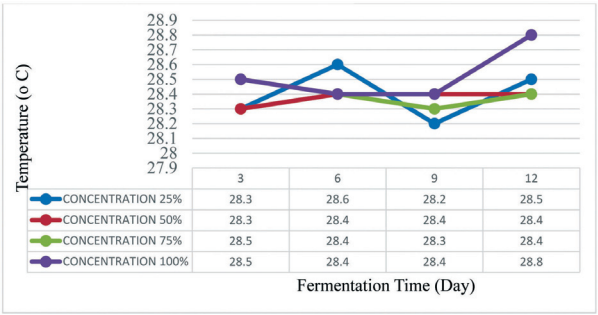


Fig. 4. Fluctuating temperatures during fermentation

Temperature

Figure 4, in this study the temperature value were in the range of 28.2 to 28,8 °C corresponding to the optimum temperature for most varieties of yeast, which was about 26 to 29 °C (Wignyanto *et al.*, 2006), or about 20 to 30 °C (Sari, 2009).

Reducing sugar

Figure 5, in this study the beginning sugar concentration before fermentation was 8.66% and then decreased dramatically on day 3th, the start time of fermentation. In this study, total sugar concentration of the substrate quite a bit, 8.66%. Concentration of 25% fruit waste's extract had the lowest sugar concentration than other concentration, seen on day 3th which only had the lowest residual reducing sugar than other concentrations, 1.88% so that the amount of ethanol produced in this concentration was quite low with an average 1.67% (Figure 2). It was different from the concentration of 100% fruit waste's extract that had the highest sugar in this study, which was 8.66% so the amaunt of ethanol produced higher than the concentration of fruit waste's extract others, 6.9% (Figure 2).

On day 12th, remaining reducing sugar concentration that has not been consumed by *Saccharomyces cerevisiae* at concentrations of 25%, 50%, 75%, and 100% fruit waste's extract, was 0.08%; 0.41%; 0.81%; and 0.92%.

According to ANOVA test, the treatment of the variations of fruit waste's extract concentration effected ethanol's amount, because the significant value was less than 0.05, which was 0.00. The maximum concentration in 100 % fruit waste's extracts (6.9%).

Effect of the variation of the length of fermentation timeto ethanol's amount with *Saccharomyces cerevisiae*

The amount of ethanol produced by variations of the length of fermentationtime of fermentation found in Figure 6. On day 3th to day 9th, the ethanol's amount continues to increase, which was 2.79% to 5.15%.

In this study, day 9th was the optimum time of fermentation ethanol in accordance with the optimum fermentation time as the result ofof

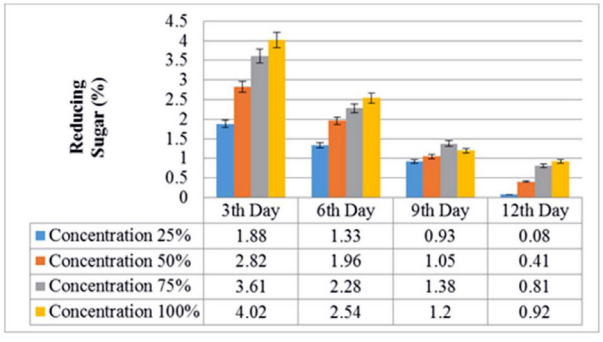


Fig. 5. The concentration of reducing sugars (%)

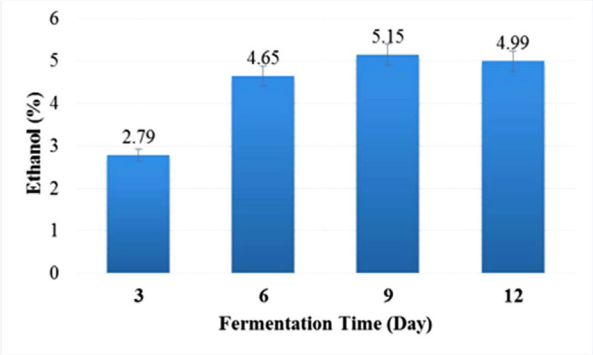


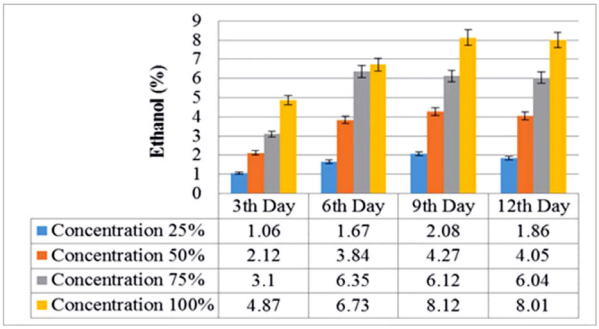
Fig. 6. The average of ethanol's amount (%) to variations of the length of fermentation time (days)

research of Jumari *et al.* (2009) and Kurniawati (2009). But after an increase, ethanol concentration decreased on day 12th, 4.99%. The concentration of sugar in the substrate of 100% fruit waste's extract was 8.66%. The sugar concentration was quite a bit to be used *Saccharomyces cerevisiae* to ferment ethanol on day 12th so that the amount of ethanol produced had decreased.

Significant value from ANOVA test was 0.000. That values obtained was significantly smaller than 0.05. This indicates that the variation of the length of fermentation time affected ethanol's amount from fermentation proses with *Saccharomyces cerevisiae*. The optimum of fermentation time is on the 9th days (5.15%).

Effect of combination of variations of PIOS fruit waste's extract concentration and variation of the length of fermentation time to ethanol's amount with *Saccharomyces cerevisiae*

According to ANOVA test, the significant value was 0.000. Values obtained significantly smaller than 0.05. Figure 7, shows that concentration of 100% fruit wastes extract was the maximum substrate so can



	3th Day	6th Day	9th Day	12th Day
Concentration 25%	1.06	1.67	2.08	1.86
Concentration 50%	2.12	3.84	4.27	4.05
Concentration 75%	3.1	6.35	6.12	6.04
Concentration 100%	4.87	6.73	8.12	8.01

Fig. 7. The average ethanol's amount (%) based on the combination treatment between the variations of fruit waste's extract concentration (%) and fermentation time (days)

produce a high ethanol (Amalia *et al.*, 2014) and fermentation time day 9th was the optimum fermentation time (Jumari *et al.*, 2009) to produced ethanol, which was 8.12%. Therefore, it can be concluded that the maximum concentration was 100% fruit waste's extract within the optimum 9th days fermentation (8.12%).

DISCUSSION

Effect of the variations of PIOS fruit waste's extract concentration to ethanol's amount with *Saccharomyces cerevisiae*

The composition of substrate that were used in this are shown were in Figure 1, determine the contained components in the substrate. Based on Figure 2, it can be seen that the 100% substrate concentration of fruit waste can produce the highest ethanol amounts, 6.9%. In more substrate, the glucose was converted by enzymes increased compared to lower substrate (Amalia *et al.*, 2014). Microbial growth gets better at 100% concentration fruit waste's extract for more nutritional needs are met and allows ethanol to be converted was also increased. This shows that concentration of 100% fruit waste's extract was the maximum concentration, so the ethanol amounts which produced was highest.

Kunaepah (2008) explore there were many factors affecting fermentation include substrate, temperature, pH, oxygen, and microbes. Several factors are used as supportive data in this study, such as pH, temperature, and reducing sugar.

pH

Figure 3, in this study the pH value were in the range of 4.11 to 4.59 whichin accordance with the conditions of pH optimum of *Saccharomyces cerevisiae*, 4 to 5 (Hidayat *et al.*, 2006). That conformity was one of the important factors that affect the growth of microorganisms and the formation of the product in the fermentation process because each microorganism had an optimum pH range (Nugroho *et al.*, 2008). If the pH value is not appropriate, it will be a limiting factor in the fermentation process.

Temperature

Figure 4, in this study the temperature value were in the range of 28.2 to 28.8 °C corresponding to the optimum temperature for most varieties of yeast, which was about 26 to 29 °C (Wignyanto *et al.*, 2001), or about 20 to 30 °C.

Reducing sugar

The beginning sugar concentration before fermentation was 8.66% and then decreased dramatically on day 3th happens because of the reducing sugar which contained in the medium was used as carbon for the yeast cells to synthesize energy through ethanol fermentation (Putri and Sukandar, 2008).

In this study, total sugar concentration of the substrate was quite a bit, 8.66%. That showed the ability of *Saccharomyces cerevisiae* had not been maximal to break down the sugars into ethanol, that's causing the ethanol amounts converted low in this study (Winarti, 1996). Based on this, it was known that the availability of sugar was limiting factor in the fermentation process of ethanol. On day 12th, remaining reducing sugar concentration that has not been consumed by *Saccharomyces cerevisiae* at concentrations of 25%, 50%, 75%, and 100% fruit waste's extract, was 0.08%; 0.41%; 0.81%; and 0.92%. The content of the substrate fruit waste's extract may still contain many components of oligosaccharides. Yeast must first produce enzymes to break down the system components oligosaccharides and the disaccharide fruit waste's extract into simpler sugars. The simple sugar content in the fruit waste's extract that will facilitate *Saccharomyces cerevisiae* to be consumed (Rahim, 2009).

According to ANOVA test, the treatment of the variations of fruit waste's extract concentration effected ethanol's amount, because the significant value was less than 0.05, which was 0.00. The maximum concentration in 100 % fruit waste's extracts (6.9%).

Effect of the variation of the length of fermentation timeto ethanol's amount with *Saccharomyces cerevisiae*

The amount of ethanol produced by variations of the length of fermentationtime of fermentation found in Figure 6. On day 3th to day 9th, the ethanol's amount continues to increase. This was caused by fermentation process, the number of microbes was affected by the length of fermentation time, which is longer fermentation time then the number of microbes more and higher ethanol production (Suri *et al.*, 2013).

In this study, day 9th day was the optimum time of fermentation ethanol in accordance with the optimum fermentation time as the result of research Jumari *et al.* (2009)and Rahim (2009). But after an increase, ethanol concentration decreased on day 12th, 4.99%. That was because the longer the fermentation time, the nutrients in the medium decreases with increasing the number of cells that can lead to competition and will eventually enter the death phase (Kusumaningati *et al.*, 2013). The concentration of sugar in the substrate of 100% fruit waste's extract was 8.66%. The sugar concentration was quite a bit to be used *Saccharomyces cerevisiae* to ferment ethanol on day 12th so that the amount of ethanol produced had decreased, because S*accharomyces cerevisiae* had the ability to optimally convert sugars to ethanol at the beginning of 10% reduced sugar (Wignyanto *et al.*, 2001). Therefore, can be optimization efforts to increasing amount of ethanol produced, for example by adding nutrients to the substrate.

Significant value from ANOVA test was 0.000. That values obtained significantly smaller than 0.05. This indicates that the variation of the length of fermentation time affected ethanol's amount from fermentation proses with *Saccharomyces cerevisiae*. The optimum of fermentation time is on the 9th days (5.15%).

Effect of combination of variations of PIOS fruit waste's extract concentration and variation of the length of fermentation time to Ethanol's amount with *Saccharomyces cerevisiae*

According to ANOVA test, the significant value was 0.000. Values obtained significantly smaller than 0.05. This indicated that the the combination treatment between the variations of fruit waste's extract concentration and fermentation time effected ethanol's amount.

Based on Figure 7, it shows that concentration of 100% fruit wastes extract was the maximum substrate so can produce a high ethanol (Amalia *et al.*, 2014) and fermentation time day 9th was the optimum fermentation time (Jumari *et al.*, 2009) to produced ethanol, which was 8.12%. Therefore, it can be concluded that the maximum concentration was 100% fruit waste's extract within the optimum 9th days fermentation (8.12%).

In this study, the result is as follows: the treatment of fruit waste's extract concentration effected ethanol's amount, the maximum concentration in 100 % fruit waste's extract (6.9%); the treatment of fermentation time effected ethanol's amount, the optimum of fermentation time on the 9th days (5.15%), and the combination treatment between the

variations of fruit waste's extract concentration and fermentation time effected ethanol's amount, the maximum 100% fruit waste's extract within the optimum 9th days fermentation (8.12%).

Therefore, further research is required of Total soluble sugar and protein content of the samples of fruit extract waste to get optimum ethanol content. Total soluble sugar and protein content of the samples were estimated by the standard method. If we know the total soluble sugar from every fruit, we can add fruit waste with high sugar content for example pineapples as a raw material (Vaitheki and Deepa, 2016).

In many studies, the agri-industrial waste substrates were suplemented with further nutrients such as glucose, and/or nitrogen sources such as yeast extract or inorganic sources such as ammonium sulphate or sodium nitrate. Other supplements included mineral salts and trace elements. Supplementation with wheat bran is also common. The extent of supplementation is influenced by the substrate characteristics, as well as the growth requirements of the microorganism used. Where fruit-processing waste is used, the substrate may contain many of the minerals required, as well as residual sugars, and will therefore require less or no supplementation (Zheng and Shetty, 2000). A viable alternative is the supplementation of the fruit-processing wastewater with solid waste to effect a bioremediation–beneficiation result (Khan *et al.,* 2015). Another method that can also be applied is to use *Aspergillus* sp. to do hydrolysis because *Aspergillus* sp. has a potential ability to produce cellulase enzyme in hydrolysis process to produce ethanol (Khan *et al.,* 2015 and Kusumaningati, 2017). Enzymatic hydrolysis was carried out at the optimum temperature (Hossain, 2015).

CONCLUSION

In this study, the conclusion as follows Fruits waste had a low potency as a raw material for bioethanol, so further research is required on additional glucose content or supplement in substrate of fruit extract waste for optimum product.

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