Utilization of solid tapioca waste for bioethanol production by co-fermentation of baker’s and tapai yeast

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Abstract. Tapioca processing from cassava produces abundant solid wastes. If left untreated, it potentially increases greenhouse gas emissions from the decomposition of organic matter. These gas emissions are known to accelerate global climate change. Tapioca solid waste (TSW) has high starch and cellulose content, making energy recovery possible through conversion to bioethanol. This research aimed to produce bioethanol from TSW by a co-fermentation method of baker’s and local tapai yeast. The research was conducted through multi-stages of enzymatic hydrolysis, followed by fermentation, then distillation. The hydrolysis produces hydrolyzate with a relatively high reducing sugar concentration. The ethanol fermentation results were optimally achieved in 48 h, namely substrate fermented by tapai yeast first for 24 h, followed by the addition of baker’s yeast and fermentation time to 48 h. This process produced the highest yield and bioethanol concentration, almost 2 times higher than fermentation using baker’s or tapai yeast only. This results suggested that TSW can be used for bioethanol production by co-fermentation of baker’s and tapai yeast method. TSW usage will reduce global warming, bioethanol production can be widely applied in various fields replacing fossil fuel, thus has the potential to reduce global warming and global climate change.

1. Introduction
One main cause of climate change is the release of greenhouse gases into the air from the decomposition of agro-industrial organic wastes. Various types of organic waste were generated from the agro-industrial processing, such as corn straw [1], sago frond [2], and cassava pulp [3]. Among these types of wastes, cassava pulp requires further attention due to its widespread usage from small to large industries and the very abundant usage volume. Indonesia is the fourth largest cassava producer globally after Nigeria, Thailand, and Brazil, producing around 19-21 million tons between 2015 to 2019 [4]. Cassava could be processed into various products, one of which is tapioca flour. Tapioca processing generates liquid and solid waste at around 70-80% from the raw material [5]. Known locally as "onggok", this solid waste is produced from the pulping and pressing of cassava, reaching up to 20-30%, which potentially generates solid wastes up to 4-6 million tons. This organic waste is easily decomposed and potentially worsens environmental problems. The decomposition of tapioca wastes has been reported to emit greenhouse gases such as methane and carbon dioxide [6], which trigger global warming. Hence TSW requires better handling and processing alternatives to avoid local environmental pollution and accelerating global climate change.
Climate change has currently prompted researchers, industries, governments, and communities to find ways to reduce and better manage waste. Waste reduction and reuse of organic waste will help reduce pressure on the planet's resources while reducing greenhouse gas emissions generated through organic waste decomposition. Improving the value of TSW into various types of products is an effort to address this problem. Tapioca solid waste can be used as raw material for various products since it is abundant with high carbohydrate content, especially starch, which reaches 60% [7]. Various processing methods for TSW to improve its value have been used, including biogas [8], animal feed [9], briquettes [10], and sugar reduction [11]. Another alternative is carbohydrates from TSW, which can be processed into bioethanol. Bioethanol is a clean energy source and better candidate for renewable fuel substituting fossil fuels that have been reported to reduce greenhouse gas emissions, induce global warming, and global climate change [12].

Bioethanol is generally produced through fermentation, where fermentation processes with starch hydrolyzate substrates using monocultures of various microbes have been developed [3]. However, the fermentation yield improvement using the co-fermentation method using a combination of baker’s and tapai yeast is relatively rare for TSW. Fermentation improvement was carried out on the mixing time between the baker’s yeast cultures with tapai yeast used in this experiment. The mixing time of both yeasts was thought to improve the synergy of the performance of each microbial culture to bioethanol produce. Research on bioethanol production using tapai and baker’s yeast with Sago Hampas substrate has been previously reported. However, the fermentation process was carried out without the optimization of mixing the yeast processes for a relatively long time, namely 14 days [8].

Baker’s and tapai yeast are microbial cultures that are cheap and easy to obtain. In Indonesia, tapai yeast has been known for a long time and is applied in various types of food processing and fermented drinks. Baker’s yeast is known to contain a single Saccharomyces cerevisiae culture, while tapai yeast contains multiple cultures of mold, yeast, and bacteria [13]. The combination of all these microbes' performance is expected to have synergy in the fermentation process to improve bioethanol production better. Based on these problems, this study aimed to optimally produce bioethanol from TSW by using the co-fermentation method using baker’s and tapai yeast, emphasizing the optimization in mixing-time. Changes in the substrate's initial pH, reducing sugar concentration, bioethanol concentration, and fermentation efficiency during the fermentation process were analyzed as an indicator of the performance of the production process.

2. Materials and methods

2.1. Materials and sample preparation
This research used baker’s and tapai yeast which a obtained from traditional markets; TSW was obtained from tapioca processing in Tanah Baru Bogor, West Java. Tapioca solid waste was sun-dried until the moisture content was about 12%, then milled and passed through a 40 mesh sieve.

2.2. Enzymatic hydrolysis
Tapioca solid waste of slurry flour (30% w/v) was heated at 95-100°C until the starch fraction gelatinized, then added 1.2 mL/kg α-amylase enzyme (600 U/mL), and stirred for one h then the temperature was lowered to 55°C, which then the enzymes of amyloglucosidase and cellulase were added simultaneously. This process produced glucose, which still contains waste residue and was used as a fermentation substrate for bioethanol production.

2.3. Fermentation and distillation
Fermentation was designed with variation of fermentation method (T), namely (T₁) the substrate was fermented with baker’s yeast, (T₂) the substrate was fermented with tapai yeast, (T₃) the substrate was
fermented with mixture of baker’s and tapai yeast, (T4) the substrate was fermented with tapai yeast for 24 h, followed by the addition of baker’s yeast and further fermentation for 48 h, (T5) the substrate was fermented with tapai yeast for 48 h, followed by the addition of baker’s yeast with addition of fermentation for 12 h, (T6) the substrate was fermented with baker’s yeast culture for 24 h, followed by the addition of tapai yeast and further fermentation for 48 h, and (T7) the substrate was fermented gradually with baker’s yeast for 48 h, followed by the addition of tapai yeast and fermentation for 24 h. Each treatment was made in series according to the fermentation time (0, 6, 12, 18, 24, 36, 48, 72 h) and repeated two times.

Fermentation was carried out in a batch process under anaerobic conditions in a 250 mL Erlenmeyer flask with a total working volume of 100 mL, fermentation time of 72 h, temperature 30°C, initial pH of the substrate of 5.01, initial concentration of reducing sugar 20.70% w/v. Baker’s and tapai yeast (2.5 g) were added to 10 mL of the substrate and incubated for 24 h for cell propagation and used as a starter culture. A substrate of 90 mL was added to the yeast starter culture according to the treatment. Substrate fermentation using only baker’s or tapai yeast was added with a starter culture of 10 mL, while fermentation by mixing the baker’s yeast culture with tapai yeast simultaneously or gradually was added to each starter culture of 5 mL. Furthermore, the fermented substrate was distilled at 90 ± 5 °C to produce bioethanol. Sugar content reduction, pH, and bioethanol content were measured every 24 h until 72 h.

2.4. Data analysis
The fermentation process parameters were measured, namely bioethanol content, sugar content reduction, substrate pH, and bioethanol production efficiency [14]. The data obtained were analyzed for their mean values and presented in graphical form.

3. Result and discussion

3.1. Characteristics of Tapioca Solid Waste
Based on the measurement, the means (± standard deviation) of dry TSW moisture content was 12.32 ± 0.78%, protein 0.94 ± 0.77%, fat 1.07 ± 0.02%, ash 0.79 ± 0.01%, and carbohydrates (by difference) 84.88 ± 0.80%. The carbohydrate fraction consists of 60% starch and 24% crude fiber. This relatively high starch content had high potential to be hydrolyzed into glucose and used as bioethanol. The solid waste content of tapioca in this study was relatively similar to the previous study, which contained starch 60.1% [7]. However, it was smaller than another similar study, namely 68.30% [3].

3.2. Bioethanol concentration
The treatment of various types of co-fermentation methods resulted in different concentrations of bioethanol. From measurement, the co-fermentation method between the baker’s and tapai yeast cultures tends to produce a higher concentration (5.75-7.18%) than the mono-culture fermentation method (4.06-4.39%). The T4 treatment resulted in relatively higher bioethanol concentrations in comparison with other treatments. This concentration is produced from the co-fermentation mechanism, first, the substrate was fermented with tapai yeast culture for 24 h, followed by the addition of tapai yeast culture and fermented for up to 48 h that produced a bioethanol concentration of 7.18 ± 0.58%, an additional fermentation time of up to 72 h tends to decrease the bioethanol concentration to 6.01 ± 0.37%. This result was not significantly different from the bioethanol concentration from the T3 treatment, which was 7.09 ± 0.39%. However, the fermentation time needed was longer, namely 72 h.

In general, Figure 1a showed that the rate of bioethanol concentration increased rapidly until the fermentation time was 24 h, then the bioethanol concentration increased slowly and reached a peak at 48 h. It was due to the increasing concentration of produced bioethanol and acid, while the substrate concentration decreased. Ethanol can inhibit the fermentation process through product inhibition.
mechanism, while acid can reduce the substrate's pH so yeast could not grow optimally. The bioethanol concentration in this study was relatively similar to previous study, which is 6.5% [1]. However, it was relatively lower than the bioethanol concentration of sago hampas hydrolyzate fermented with baker's and tapai yeast, which was 12.01% [8].

![Figure 1. Changes in (a) bioethanol concentration and (b) reducing sugar on various types of fermentation treatments method](image)

### 3.3. Reducing sugar

The initial concentration of reducing sugar in the fermentation process was 20.07%. The concentration of reducing sugar in the mono-culture fermentation treatment (T₁ and T₂) decreased by about 48-49%. A more significant decrease occurred in the co-fermentation treatment (T₄, T₅, T₆, and T₇) in the range of 78-85%. This result showed that the microbes in the co-fermentation treatment consumed the substrate higher than the mono-culture fermentation. Figure 1b showed that the reduction rate of reducing sugar concentration occurs faster in the initial phases (24 h) then the rate slows relatively. Reducing sugar concentration negatively correlated with bioethanol concentration, namely a decrease in the reduction in sugar concentration, followed by an increase in bioethanol concentration in the fermentation process.

### 3.4. pH

The co-fermentations method showed that the initial pH of the substrates decreased during the fermentation process. The initial pH decreased drastically within 24 h, then the change was relatively stable, as shown in Figure 2a. The co-fermentations method (T₆, T₇, T₈, and T₉) between the baker and tapai yeast tended to produce a lower substrate pH compared to the non-co-fermentations treatment (T₁ and T₂). The decrease in the substrate's pH indicated that an acidic compound had been produced during the fermentation process [14]. All treatments showed a positive correlation between reducing sugar concentration and pH, namely a decrease in the total sugar concentration, followed by a decrease in the substrate's pH. Decreased substrate pH causes the effectiveness of microbial performance to bioethanol produce to decrease [14]. This result had been confirmed by the decrease in bioethanol concentration after the substrate pH reaches around 3.0-3.5. Bioethanol production by fermentation optimally has been reported to range between 5.0-7.5, depending on the type of microbe [15].
3.5. The efficiency of bioethanol production

The efficiency of bioethanol production using the co-fermentation method in this study was determined based on bioethanol products' comparative efficiency with reducing sugar substrate, substrate consumption, fermentation efficiency, and yield [14], as shown in Figure 2b. In general, the treatment with the baker’s yeast co-fermentation method with tapai yeast resulted in higher efficiency of using the substrate and fermentation compared to the mono-culture fermentation method. Treatment T4 had substrate consumption and fermentation efficiency of 79.33% and 67.98% respectively, relatively similar to the T5 treatment, namely 78.59% and 67.18%. However, T4 treatment tended to produce a higher bioethanol concentration (7.18 ± 0.58%) faster (48 h). Meanwhile, T5 treatment produces bioethanol with a 7.09 ± 0.39% concentration, which takes up to 72 h. The bioethanol concentration in Figure 1a confirmed the result. Fermentation efficiency of 67.98% (T4) proved that the reducing sugar consumed by microbes had been converted into bioethanol by 67.98%, and the rest (32.03%) was used for other processes to maintain cell metabolism, forming biomass or organic acids. The fermentation efficiency in this study was relatively higher compared to previous study which was 45.10% [7]. However, it was lower than that of Srinorakutara's research which reached 91.00% [16]. The T6 and T7 treatments had relatively high substrate efficiency, namely 81.64% and 85.30%, respectively, but the fermentation efficiency was relatively low, namely 56.99% and 55.80%. This result was due to the concentration of reducing sugar in the substrate not being converted optimally to bioethanol.

The yield was determined based on the percentage of the ratio between bioethanol volume and dry TSW flour weight (% v/w). The T4 and T5 treatments produced relatively similar yields, namely 23.92% and 23.05%, respectively. This result was because the treatment tends to produce the highest bioethanol concentration than other treatments (Figure 1a). These results indicated that to produce 1 L of bioethanol, it took about 4 kg of dry TSW. Several researchers had reported that there are differences in the yield value of bioethanol production from solid waste tapioca, namely 33-54% [17], 32.40% [18], and 53.00% [19]. The difference in yield value was caused by different types of microbes, conditions, and fermentation process methods in bioethanol production.

4. Conclusion

This study suggested Tapioca Solid Waste, which can be used as raw material for bioethanol production. The co-fermentation method between baker's and tapai yeast yielded higher bioethanol concentration.
(7.09-7.18%), fermentation efficiency (67.18-67.98%), and yield (23.05-23.92%) compared to the mono-culture fermentation method. Thus, in the future TSW potentially can be converted into bioethanol as a renewable energy source. With further optimization of fermentation processes of TSW and high volume of annual TSW produced, this TSW utilization into bioethanol had high potential to reduce the release of greenhouse gas emissions in Indonesia, thus reducing the cause of global warming and climate change.

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