Production of Bioethanol from *Colocasia esculenta (L.) Schott* (Talas Liar) by Hydrolysis Process

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Abstract. Wild taro tubers (*Colocasia esculenta (L.) Schott Var. Antiquorum*) contain 70-80% (wt%) of starch, thus serve as potential feedstock for glucose production, providing raw materials for bioethanol production. The tubers used in this study was collected from Padang city, Indonesia. The aim of this research was to find the optimum of bioethanol yield through variation of acid catalyst (HCl, H$_2$SO$_4$ and HClO$_4$), acid concentration (0.00 N; 0.05 N; 0.10 N; 0.15 N; 0.20 N), enzyme (α-amylase, glucoamylase), enzyme volume (0.308 ml; 0.74 ml), hydrolysis time (1 hour, 2 hours, 4 hours), and fermentation time (48 hours, 72 hours, 96 hours, 122 hours, 144 hours). At varied acid catalyst usage, the highest glucose content (27.54%) was obtained by using HClO$_4$ acid with a concentration of 0.10 N and the lowest glucose level of 16.64% was obtained from the usage of H$_2$SO$_4$ acid with a concentration of 0.10 N in the hydrolysis process with temperature of 120°C for 45 minutes. The highest bioethanol content as 19.10% was obtained at the time of fermentation of 96 hours with the usage of Sacharomyces cerevisiae enzyme. The highest glucose content of 20.35% was obtained by using glucoamylase enzyme of 0.308 ml.

1. Introduction

The increasing demand for fossil fuels had took place since the Industrial Revolution as the world population grew and many countries are industrialized. Enormous combustion of the fossil fuels would contrarily increase the concentration of greenhouse gas in the atmosphere resulting in greenhouse effect and global warning. In order to prevent the air pollution from burning fossil fuel and energy crisis, many countries begin to develop renewable energy source [1]. Considerable attention has been focused on biomass as an alternative energy biosource not only for them to protect the environment, but also to meet the energy demand instead of fossil fuels [2]. The interest of biofuel (biomass based fuel) production was escalated dramatically in order to replace the fossil based fuel [3]. Biofuel, such as bioethanol, biodiesel and biogas, has become a feasible and economical solution. Among them, bioethanol is by far the most widely used biofuel for transportation worldwide. Production of bioetanol from biomass is one way to reduce both consumption of fossil fuels and environmental pollution [4]. Bioethanol is the product of the sugar fermentation process from carbohydrate sources [5]. The technology of bioethanol production from biomass feedstocks consists of several steps, and varies depending on the type of raw materials used. It becomes more sophisticated as the raw materials turn from sugars and starches to cellulosic materials. Unlike starch, the specific structure of cellulose favors the ordering of the polymer chains into tightly packed, highly crystalline structures that is water-insoluble and resistant to depolymerisation [6]. However, production of bioethanol from starch and...
sugars involving hydrolysis and fermentation followed by separation/purification, differ from cellulose feedstocks that require pretreatment [7]. Hydrolysis of raw materials include the processing steps that convert the carbohydrate polymers e.g. cellulose or starch into monomeric sugars. Cleavage of these polymers can be catalyzed by enzymes or chemically by acids. The reaction can be described as follows:

\[
(C_6H_{10}O_5)_n + nH_2O \rightarrow n(C_6H_{12}O_6)
\] (1)

Moreover, the fermentation steps involving the chemical transformation of organic substances into simpler compounds by the action of enzymes. The fermentation reaction is caused by yeast or bacteria which feed on simple sugars [8]. The simplified reaction equation is:

\[
\text{Fermentation} \quad C_6H_{12}O_6 \rightarrow 2C_2H_5OH + 2 CO_2
\] (2)

There are three types of bioethanol feedstocks: (a) sucrose-containing feedstocks (e.g. sugar beet, sweet sorghum, and sugar cane), (b) starchy materials (e.g. wheat, maize and barley), and (c) lignocellulosic biomass (e.g. wood, straw, and grasses). All of these materials can be utilised for bioethanol production [9]. In this context, some researchers have conducted studies on the use of banana and pineapple wastes, cassava and sweet potato peels, papaya waste, corn cobs, jackfruit seeds, and coffee husks [10-14].

Taro (Colocasiaesculenta (L.) Scott.), a member of Araceae family, is a plant cultivated mainly in tropical and subtropical regions such as Asia, Oceania and Africa. In Asia, taro is used to produce variety of desserts, snacks and confectionary products, leaving huge chunks of taro peel [15]. Wild taro tubers contain considerable amount of starch (70-80 g/100 g dry taro) [16]. The high starch content of taro serves as a great feedstock for glucose production, providing raw materials for bioethanol production [17]. Braide and Nwaoguikpe produced the maximum of 12.90% of bioethanol from wild taro through enzyme hydrolysis followed by \textit{Saccharomyces uvarum} fermentation process [18]. Adegunloye and Udenze reported the effect of using \textit{Aspergillus niger} and \textit{Saccharomyces cerevisiae} on wild taro peels fermentation to produce bioethanol [19].

Though few works has been conducted using wild taro tubers for bioethanol production, it is yet to be determined which hydrolysis process (acid or enzyme hydrolysis) would give the highest yield of ethanol and the condition under which an optimum yield can be achieved. Thus, the knowledge would provide information required in the industrial production process design. Therefore, this research aims to establish an effective process to produce bioethanol from wild taro tubers using bread yeast (\textit{Saccharomyces cerevisiae}) by comparing acid and enzyme hydrolysis pathway. Hydrolysis parameters (e.g. hydrolysis time, types of acid or enzyme catalysts used, acid concentration, enzyme loading amount) in addition to fermentation parameters (e.g. fermentation time) were also evaluated.

2. Experimental section
2.1. Materials preparation
Wild taro tubers (Colocasiaesculenta) used in this study, as shown in fig. 1, were collected from Padang city, Indonesia. Prior to use, the cleaned tubers were soaked in NaCl (1% w/v) at a ratio of 2:1. 500 g of tubers for each experiment was steamed then dried inside the oven at 60°C for 24 hours. The tubers were blended and sieved to produce taro flour.

![Figure 1. Taro (Colocasia esculenta) Plant and Tuber](image)

2.2. Fermentation process
For acid hydrolysis, 20 g of taro flour mixed with acid catalysts (HCl, H₂SO₄ or HClO₄) with various concentrations (0 N, 0.05 N, 0.10 N, 0.15 N and 0.20 N). The mixture was refluxed at 120°C for certain hydrolysis time (1 hours, 2 hours or 4 hours) then cooled and filtered. The hydrolysis solution was added with NaOH 1 N to adjust the pH into 4. The same process was repeated for enzyme hydrolysis, except for two commercial enzymes (bacterial β-amylase and glucoamylase) was used instead of acid, obtained from Sigma-Aldrich, Germany. The loading amount of enzyme was also varied (0.308 mL and 0.74 mL).

Afterwards, the yeast (Saccharomyces cerevisiae) purchased from local shop in Padang city, was mixed into the hydrolysis solution at a ratio of 1:3. The mixed solution was introduced into the fermentation reactor. The fermentation process was carried out anaerobically for certain fermentation time (48 hours, 72 hours, 96 hours, 122 hours, 144 hours). The fermented product was filtered and then fed into the distillation flask, the temperature is kept at 80°C. Distillation process was carried out until bioethanol stops dripping.

![Flow Diagram of Bioethanol Production](image)

**Figure 2.** Flow Diagram of Bioethanol Production

### 2.3. Analysis

The starch content of taro was analyzed by using Luff-Schoorl method. 2 g of taro flour was mixed with 100 mL of HCl 3% (v/v) and heated for 3 hours. The mixture was cooled and neutralized by adding NaOH 3% (w/v) then filtered. 25 mL Luff-Schoorl solution was added to 10 mL of the filtrate followed by 15 mL of deionized water. The mixture was heated for 10 minutes and cooled immediately in cold water. 25 mL of H₂SO₄ 25% (v/v) and 15 mL of KI 20% (w/v) were added slowly to prevent excessive foaming. Titrate with Na₂S₂O₃ 0.1 N until a dull yellow colour appeared, the starch indicator was added and the titration was completed until blue colour disappeared. The same procedure was also conducted to analyze glucose content of hydrolysis products.

### 3. Results
3.1. Hydrolysis process

3.1.1 Acid hydrolysis
The yield of glucose produced from acid hydrolysis steps is shown in figure 3.

![Figure 3. Glucose Yield at Various Acid Catalysts Concentration](image)

As shown in Figure 3, concentration above 0.1 N and 0.15 N are accompanied with lower glucose yield. For H₂SO₄ and HCl, the maximum glucose yield was obtained at acid concentration of 0.15 N. However, the maximum glucose yield for HClO₄ catalysts was obtained at concentration of 0.1 N. Degradation of starch during hydrolysis step is a critical problem for acid processes because starch degradation also generates fermentation inhibitors that can inhibit the fermenting microorganisms during the downward fermentation process, thus jeopardizing starch to ethanol conversion yield and the overall biomass-to-ethanol conversion efficiency. The highest glucose yield of 20.15% was obtained using HClO₄ 0.1 N as catalysts.

3.1.2 Enzyme hydrolysis
Figure 4 shows that as enzyme loading amount increase the total glucose produced also increase. This behavior caused by higher growth rate of microorganisms at high values of inoculums concentrations which lead to higher rate of starch degradation in the process. However, glucoamylase produce glucose faster than α-amylase. After 60 minutes of hydrolysis process, glucoamylase and α-amylase generates 20.35% and 6.12% of glucose yield, respectively. This result caused by the nature of enzyme behavior. The α-amylases are endohydrolases that randomly cleave α-1,4 glycosidic bonds of linear amylase and branching amylopectin chains of the starch molecule [20]. Contrarily, glucoamylases are exoamylases that cleave both α-1,4 and α-1,6 glycosidic bonds from the non-reducing end of starch molecules [21], thus producing glucose faster than α-amylase.
The highest yield of glucose with 20.35% conversion was obtained from enzymatic hydrolysis as compared with 20.15% conversion from acid hydrolysis. The result suggesting that enzymatic hydrolysis is a suitable method compared to acid hydrolysis under the conditions of this study. This has been attributed to the decrease of sugar content by degradation of glucose to furfural and Hydroxy Methyl Furfural (HMF) during acid hydrolysis or conversion of glucose to levulinic, acetic or formic acids. These substances are toxic to yeast and thus can inhibit its growth by decreasing the intracellular pH of the fermenting medium, resulting in the death of microorganisms [22].

### 3.2. Fermentation process

The effects of fermentation time on bioethanol fermentation was determined by measuring the bioethanol concentration after 48, 72, 96, 120 and 144 hours. The result is presented in figure 5.

![Bioethanol Production Over Time](image)

**Figure 5.** Bioethanol Production Over Time

The longer fermentation time, the higher bioethanol concentration obtained. However, after 96 hours the concentration decreased significantly. In the first stage of fermentation process, the bioethanol increase from 6.0% at 48 hours to 10.24% at 72 hours. The highest bioethanol concentration, which is 19.1%, obtained at 96 hours of fermentation process. This concentration decreased to 16.94% at 120 hours and after 144 hours of process the bioethanol concentration decreased to 7.24%. The results suggesting that after 96 hours the carbon source had reduced and the number of microorganisms present reduced as well, thus resulting in lower yield of bioethanol. This result is in correlation with
Ishmayana et al. that after 120 and 144 hours of fermentation time the viable cell number of yeast was decreased [23].

The comparison of bioethanol production from wild taro in this study with other feedstocks can be seen in table 1. Comparative studies of bioethanol production from different feedstocks shows that wild taro tuber has higher efficiency than other biomass. The process is cost effective, thus could potentially be developed as the feedstock of bioethanol production on a large scale.

Table 1. Comparison of Bioethanol Production from Wild Taro with other Raw Materials

| Raw materials        | Hydrolysis process | Bioethanol yield (%) | References |
|----------------------|--------------------|----------------------|------------|
| Wild taro tuber      | Enzyme hydrolysis  | 19.1                 | This study |
| Jackfruit seeds      | Acid hydrolysis    | 57.94                | [5]        |
| Banana peel          |                    | 7.45                 |            |
| Plantain peel        | Enzyme hydrolysis  | 3.98                 | [10]       |
| Pineapple peel       |                    | 8.34                 |            |
| Cassava peel         | Enzyme hydrolysis  | 17.6                 | [11]       |
| Sweet potato peel    |                    | 9.3                  |            |
| Papaya waste         | Enzyme hydrolysis  | 3.83 – 5.19          | [12]       |
| Corns of wild taro   | Corns of wild taro | 12.9                 | [18]       |
| Wild taro peels      | Enzyme hydrolysis  | 5.65                 | [19]       |

4. Conclusions
Bioethanol production from wild taro tubers (Colocasia esculenta) was performed by hydrolysis followed by anaerobic fermentation. The highest glucose yield of 20.15% from acid hydrolysis was obtained using HClO₄ 0.1 N as catalysts. However, glucoamylase able to produce 20.35% of glucose after 60 minutes of hydrolysis process, indicating that enzymatic hydrolysis is a suitable method compared to acid hydrolysis under the conditions of this study. Moreover, the fermentation process was able to recover maximum of 19.1% bioethanol after 96 hours of fermentation time. The results showed that high carbohydrate content in taro tubers could potentially be developed as the feedstock of bioethanol production on a large scale.

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