Letters in Applied NanoBioScience
Open-Access Journal (ISSN: 2284-6808)

Article
Volume 12, Issue 4, 2023, 148
https://doi.org/10.33263/LIANBS124.148

Enzymatic Hydrolysis of Stinky Bean (*Parkia speciosa*) Peel as Bioethanol Feedstock Preparation

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Scopus Author ID 57189692080
Received: 2.08.2022; Accepted: 3.10.2022; Published: 17.11.2022

Abstract: Stinky bean (*Parkia speciosa)*, one of Indonesia's familiar plants, was used in this study for bioethanol synthesis due to its potential starch and cellulose content. Stinky bean peel was utilized in this research as raw material because all this time, this peel was being wasted and unused. This study aims to convert stinky bean peel into glucose as a bioethanol feedstock via an enzymatic hydrolysis reaction. Stinky bean peel was crushed into powder and reacted with water using two enzymes: amylase and glucoamylase. The hydrolysis product was analyzed using a Brix refractometer to observe its glucose content. Using 10 ml of -amylase and glucoamylase enzymes with a volume ratio of 1:1 could speed up their hydrolysis kinetics to 64,85% of conversion. This highest result showed a 13,1% Brix glucose concentration from refractometer analysis, yielding 21,615 grams of glucose from 15 grams of stinky bean peel. Because of its effective performance during the reaction, the hydrolysis of stinky bean peel using -amylase and glucoamylase enzymes could be a new route to prepare raw material for bioethanol synthesis from a type of cellulose waste.

Keywords: amylase; bioethanol; enzyme; glucose; hydrolysis; *Parkia speciosa*.

1. Introduction

Agricultural industries produce large quantities of residues/waste annually, which can cause widespread environmental concerns [1]. However, agricultural waste is abundant in bioactive compounds that can be used as raw material for the production of bioethanol [2], biodegradable polymers [3], and adsorbents for the removal of contaminants [4–7], and so on. The stinky bean was a widely cultivated tropical plant in Southeast Asian nations like Indonesia, Malaysia, and Thailand [8]. Indonesians typically consume stinky beans as a complement to other vegetables. In 2014, Indonesia produced approximately 230.40 tons of stinky beans, 1.93 percent of all vegetables. In 2016, Indonesia produced 194,936 tons of stinky beans; in 2017, that number increased by 9.45% to 213,261 tons [9]. The portion of the stinky bean consumed by Indonesians was the bean, while the peel was discarded and unused. There are numerous beneficial compounds in stinky bean peel, including antioxidant compounds and a high cellulose content [10]. Due to its high cellulose content, stinky bean peel has a potential carbohydrate content of between 68.3% and 68.75% [11].

The hydrolysis process could convert these long-chain cellulose compounds into glucose units [12]. Strong acid hydrolysis and enzymatic hydrolysis are two hydrolysis processes that can break off a long chain of cellulose into small units of glucose connected by

-1,4 glycosidic bonds: Strong acid hydrolysis showed great performance in cracking a long chain of cellulose. But strong acid compounds used as catalysts would damage the structure of the glucose product [13]. Furthermore, glucose products from strong acid hydrolysis need further purification to neutralize their pH for the next step of fermentation in bioethanol synthesis, resulting in a more expensive production cost [14]. Meanwhile, the enzymatic hydrolysis process was a low-cost and mild process, making it more economically feasible for bioethanol production [15]. Catalysts for enzymatic hydrolysis included enzymes such as -amylase and glucoamylase. The long chain of cellulose, especially its glycosidic bond, was broken off by the -amylase enzyme, resulting in a smaller dextrin chain [16]. The glucoamylase enzyme then has the function of producing more glucose compounds by breaking a starch bond that the -amylase enzyme left unbroken during the reaction [17].

In our previous study, stinky bean (*Parkia speciosa*) peel was converted into bioethanol by an enzymatic process utilizing *Saccharomyces cerevisiae* microorganisms [10]. The analysis of mass balance during bioethanol production from stinky bean peel was also observed before to get a large-scale perspective for plant design purposes. The objective of this study was to conduct a deep analysis of the cellulose conversion in stinky bean peel into glucose via enzymatic hydrolysis. This study has the benefit of making hydrolysis reaction investigation a starting point to gain kinetic data of the enzymatic hydrolysis for the reactor or fermenter design.

2. Materials and Methods

2.1. Material preparation.

Stinky bean peel as raw material was collected from the traditional market in Surabaya. α-amylase and glucoamylase enzymes were purchased from a domestic chemical supplier. The enzymatic hydrolysis process following the modified procedure [18] with batch reactor set up from beaker glass and thermometer as depicted in Figure 1. Firstly, the stinky bean peel was cleaned and washed with water. Then, the stinky bean peel was chopped into small sizes using a cutter and dried by sunlight to remove its moisture content. Pre-treatment of raw material by crushing stinky bean peel using a blender and sieving to get stinky bean to peel around 60 mesh [19].

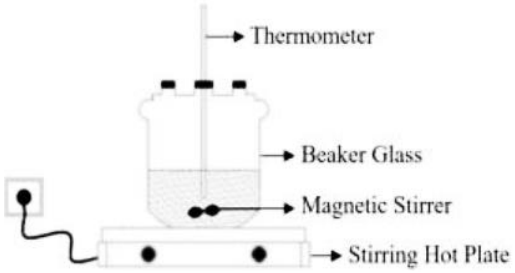


Figure 1. The schematic of a batch reactor for enzymatic hydrolysis process.

2.2. Enzymatic hydrolysis.

The enzymatic hydrolysis experiment step in this study was conducted by the same procedure as our previous research [10]. First, 15 grams of stinky bean peel particles were dissolved into 150 ml of distilled water. Then, the solution was filtered, and the filtrate was heated at 800°C in a beaker glass. The alpha-amylase enzyme was added to the filtrate solution by 2, 4, 6, 8, and 10 mL of volume. The solution was stirred vigorously at 250 rpm for 1 hour.

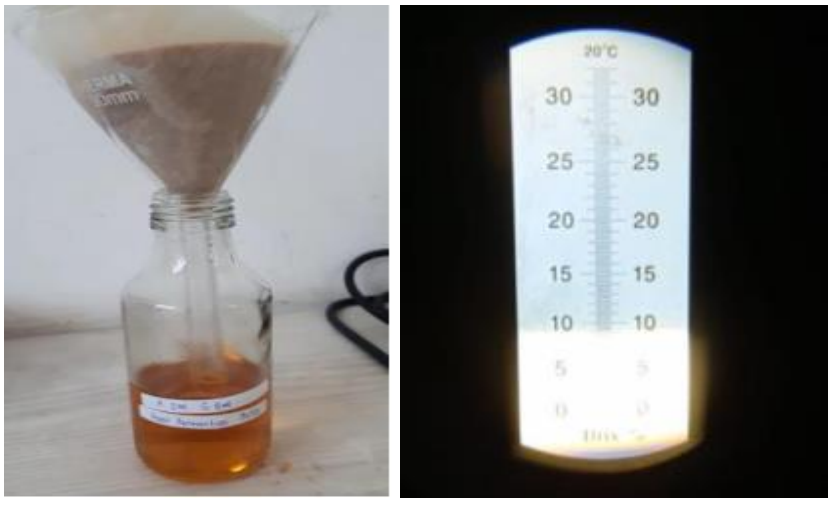
During glucoamylase enzyme addition, the solution temperature was decreased to 600°C, and the solution was mixed for 1 hour. The volume of glucoamylase addition was the same as-amylase, 2, 4, 6, 8, 10 ml, respectively. The effect of -amylase and glucoamylase addition ratios on hydrolysis conversion was investigated. Finally, glucose concentration as the main product was analyzed by a Brix refractometer. And the reacted mole of cellulose and the conversion of hydrolysis was calculated by the chemical reaction kinetics method.

3. Results and Discussion

3.1. Enzymatic hydrolysis.

The enzymatic hydrolysis of petai (*Parkia speciosa*) peel produced an orange solution with a high amount of glucose compound (Figure 2a). As mentioned before, due to the high amount of carbohydrates (68,3 – 68,75%) in petai peel waste, it can be used as one of the main resources for bioethanol production [11]. The disfigurement of lignocellulose could be the primary reason for the high efficiency of enzymatic hydrolysis to produce high glucose concentration. In addition, the fermentable sugar was produced by enzymatical hydrolysis of cellulose by cellulase enzyme (α-amylase and glucoamylase enzyme) [19]. The Brix refractometer was used for the analysis of glucose levels [20] (Figure 2b). The glucose level in the product solution was calculated from %Brix, corresponding to %mass of solute dissolving in the solution. In this research, cellulose conversion was calculated using the biochemical kinetics method due to the use of enzymes as a catalyst during hydrolysis. The mass of glucose was then divided by its molecular weight to obtain the number of glucose molecules. Conversion of enzymatic hydrolysis was calculated using Equation 1 below that, obtained from its stoichiometric reaction [21].

$$Conversion(\%) = \frac{\text{mol of glucose}}{2 \text{ x mol of cellulose}} \text{ x } 100\% \quad (1)$$



(a) **(b)**

Figure 2. (a) The resulting product of enzymatic hydrolysis; (b) Glucose concentration measurement using Brix refractometer.

The addition of both α-amylase and glucoamylase enzymes can boost the enzymatic hydrolysis reaction, which results from the increase of glucose content (Table 1). The highest glucose content (13.1 %Brix) was produced by enzymatic hydrolysis with an enzyme volume ratio of 1:1. The result of this study followed our previous research findings, while the addition

of α-amylase could enhance the product yield from mass balance analysis [10]. The α-amylase enzyme breaks off a long chain of cellulose compounds by hydrolysis of internal α-1,4-glycosidic linkages to produce a simpler dextrin chain [16]. Furthermore, the synergistic hydrolysis by adding glucoamylase enzyme will further hydrolyze the unbroken long-chain during the reaction with α-amylase [22]. The enzymatic hydrolysis reaction was performed using an optimum temperature of 80℃ for α-amylase and 60 ℃ for glucoamylase [23–25]. Both temperatures are the optimum temperature for the enzyme to reach its maximum performance due to the lowest activation energy route that happened during the reaction. The activity of α-amylase enzyme retained >80% activity at 80 ℃ and significantly reduced to 35% at higher temperatures [24]. Consequently, the higher reaction temperature will increase the reaction kinetics constant regarding the Arrhenius equation [26]. Moreover, the optimum temperature led to the broad surface area of reacted particle compound to make the reaction easier to occur [27].

Table 1. The analytical data of enzymatic hydrolysis from stinky bean peel.

α-amylase enzyme (ml)	glucoamylase enzyme (ml)	enzyme volume ratio	glucose content (%brix)	mass of glucose (grams)	mol of glucose
2	10	1:5	6,5	10,725	0,0595
4	10	2:5	7,1	11,715	0,065
6	10	3:5	10,8	17,82	0,0989
8	10	4:5	11,8	19,47	0,108
10	10	1:1	13,1	21,615	0,1199

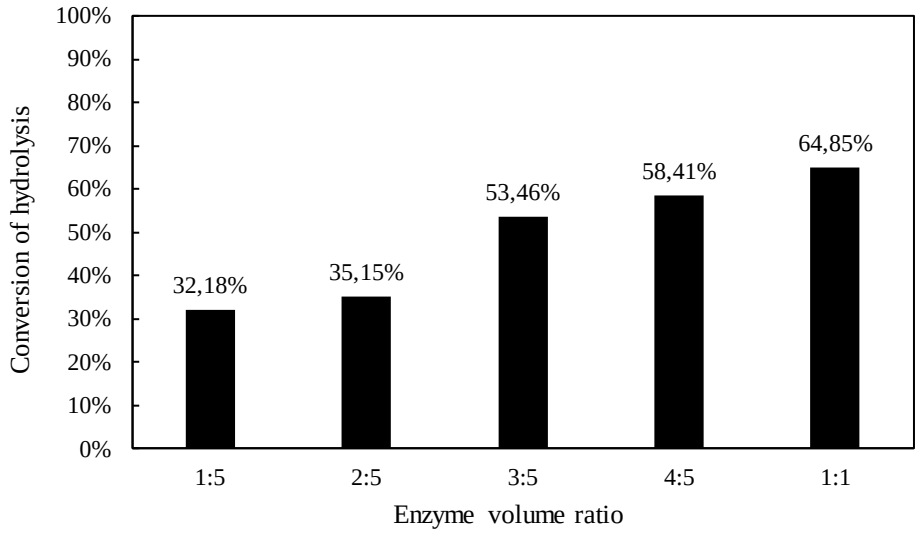


Figure 3. The effect of enzyme volume ratio with glucoamylase volume was fixed at 10 ml towards the conversion of enzymatic hydrolysis.

This study revealed that the pre-treatment process of petai peel greatly impacts enzymatic hydrolysis productivity. Transforming petai peel into a uniform particle size of around 60 mesh will increase the surface area of cellulose and enhance the molecular interaction with the enzyme [28]. A larger surface area of the reactant can reduce the mass transfer resistance of the enzyme during the reaction step [29]. Lower mass transfer resistance makes the molecular interaction occur in the bulk of fluid, called reaction regime control. Lower mass transfer resistance can increase the diffusivity of reactant molecules, and the reaction kinetics would be raised [30]. All of these factors were the reason for increasing the chemical reaction kinetics of hydrolysis producing more glucose molecules as the main product.

3.2. Conversion of hydrolysis.

The addition of α-amylase and glucoamylase enzyme in various volume concentrations has an impact on increasing glucose molecules and the single-pass conversion of hydrolysis. Generally, α-amylase and glucoamylase enzymes could act as a biocatalyst to enhance the hydrolysis reaction kinetics by reducing its activation energy [31]. Variations of α-amylase addition showed significant results after a 3:5 volume ratio (Figure 3). A small quantity of α-amylase cannot break off the long chain of cellulose yet, although the concentration of glucoamylase enzyme was made high at 10 ml of volume. Otherwise, adding glucoamylase with a fixed volume of the α-amylase enzyme causes a slight increase in hydrolysis conversion (Figure 4). These results were confirmed with our previous work, in which a low mass of resulted glucose, around 12,975 grams at 3:1 of the volumetric ratio of glucoamylase and α-amylase enzyme. These results showed that the α-amylase enzyme plays a more important role than the glucoamylase enzyme due to its activity. Based on its reaction mechanism, the α-amylase enzyme can act as a free radical that cracks off the long chain of cellulose on its glycosidic bond [32,33]. Strong acid molecules can also do this role due to their Bronsted acid site during catalysis. Nevertheless, strong acid molecules of inorganic catalysts are not suitable for bioethanol synthesis because they will increase the pH solution and can damage the molecules of produced glucose as the main feedstock of bioethanol production [34].

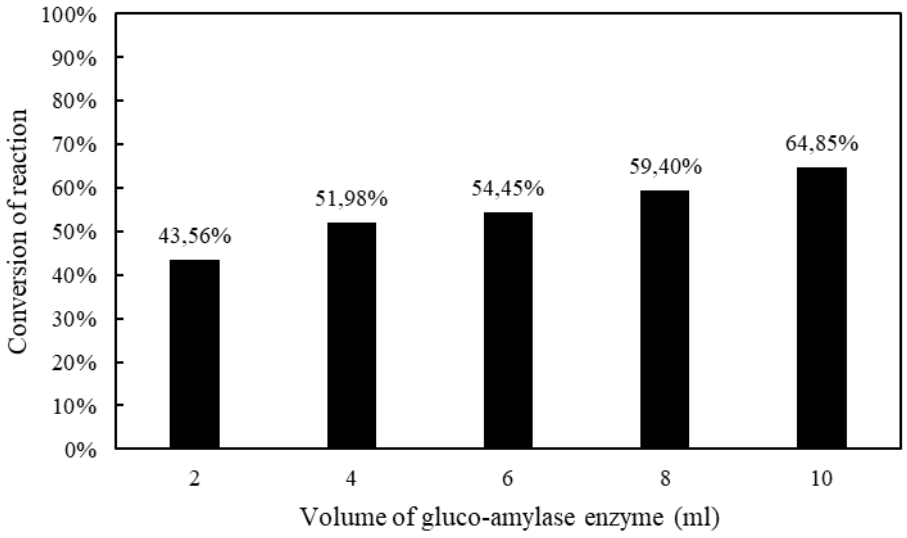


Figure 4. Conversion of hydrolysis with the variation of glucoamylase volume (ml) and fixed α-amylase volume at 10 ml.

4. Conclusions

Stinky bean was successfully converted into glucose via enzymatic hydrolysis using the amylase-based enzyme. The volumetric ratio of α-amylase and glucoamylase inside the reaction solution gives an effect to increase glucose concentration as well as the conversion of hydrolysis. The addition of α-amylase enhances the product yield by the hydrolysis of internal α-1,4-glycosidic linkages to produce a simpler dextrin chain. The synergistic hydrolysis of the glucoamylase enzyme will further hydrolyze the unbroken long-chain during the reaction with α-amylase. The addition of α-amylase and glucoamylase enzyme in various volume concentrations has an impact on increasing glucose molecules and the single-pass conversion of hydrolysis. The optimum volume ratio of cellulase enzyme 1:1 gives hydrolysis kinetics to 64,85% of conversion and the highest Brix glucose concentration of 13,1%. The optimum

temperature and the particle size of stinky bean peel were also given an important role in boosting the activity of hydrolysis. Strong acid inorganic catalyst molecules are unsuitable for bioethanol synthesis because they increase pH and damage glucose molecules, the main bioethanol feedstock.

Funding

This research was funded by Universitas Pembangunan Nasional "Veteran" Jawa Timur through the Riset Terapan scheme in 2021, the fiscal year.

Acknowledgments

This research has no acknowledgment.

Conflicts of Interest

The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

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