Direct Glucose Recovery from Cassava bagasse using Granular Starch-Hydrolyzing Enzyme

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Abstract

Biorefinery of biomass which fractionates the biomass to bio-products, is seen as the foundation of the bio-economy. Cassava is main starch source for industry in Vietnam. A long with cassava starch process, cassava bagasse was discharged at a rate of approximately 280 tons of wet bagasse (85% moisture) per every 300 tons of cassava roots processed. With the residue starch estimated up to 50-60%, the bagasse can be seen as a glucose material. By treatment of the bagasse using the granular starch-hydrolyzing enzyme Stargen 002 (DuPont) at 10 GAU/g dry bagasse at 50 °C, pH 3.5-5, the glucose can be totally recovered from residue starch in bagasse in one step process. The highest glucose concentration of the hydrolysate attained of 137 g/L and can be used for fermentation of desired products. Starch-free cellulose received can be used to develop cellulose materials. Those recovered products can help to improve the total value of the cassava production chain.

Keywords: *cassava bagasse, glucose recovery, Stargen 002*

1. Introduction

In Vietnam, approximately 55 % of cassava root productivity (10.35 million tons for 2015) are actually used for cassava starch production. Cassava bagasse (cassava pulp) as the waste of cassava starch industry is discharged at a ratio of 280 tons of bagasse (high moisture 85%) per every 250-300 tons of cassava roots processed, equivalent approximately to 5.8 million tons of fresh cassava bagasse discharged annually [1]. The bagasse contains high content of starch (30-50%) and celllulose (15-50 %), a minute amount of hemicellulose and lignin [1]. Dry cassava bagasse is currently used for animal feed or fertilizer with low value due to its low protein content [1, 2]. The waste with high moisture and starch poses serious threat to the environment, causing strong unpleasant smell as result of microbial degradation of its organic matters.

However, this bagasse in another hand can be converted into various high-value products to maximize the effective utilization of the biomass based on the biorefinery concept. The remaining starch in the bagasse can be recovered to improve the starch extraction efficiency or hydrolyzed to glucose hydrolysate [3, 4]. The glucose by its turn can be fermented to protein, biomolecules, organic acids, food aroma compounds, pigments… [1, 5] or biogaz [6]. Bagasse cellulose residue can be then treated for different apllications [2, 7].

To hydrolyze the remaining starch by conventional process, the cassava waste was firstly gelatinized at high temperature, then amylases were added [3]. It was estimated that 24 g reducing sugar can be received from 100 g cassava pulp after 4 h hydrolysis [4]. Recently, Stargen 002, DuPont was reported as a new amylase enzyme which could directly hydrolyze the granular starch without gelatinization step. Using this enzyme, the conversion of residue starch in bagasse to glucose can be done in one step, the process could reduce the energy input and the operation time.

The research focused on using granular starch Stargen002, DuPont to directly recover glucose from residue starch in the cassava bagasse. The effect of reaction condition was investigated and discussed.

2. Materials and methods

2.1 Materials

Fresh cassava bagasse was collected from Yen Bai Cassava Starch Company, stored at -20^{0}C for further use.

Stargen 002 was provided by DuPont company, which containing 570 glucoamylase unit (GAU)/mL

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2.2. Analysis

2.2.1. Composition of cassava bagasse

The starch content in cassava bagasse was determined using the Total Starch Kit of Megazyme (USA). Briefly, cassava bagasse was ground to pass 0.5 mm mess. An amount of 10 mg of milled bagasse was added to 0.2 mL of aqueous ethanol (80 % v/v) to wet sample and aid dispersion. Then, starch in cassava bagasse was hydrolysis using amylase (α-amylase, glucoamylase), the glucose released was measured using D-Glucose Assay Kit GOD-POD Format (Megazyme, USA)

Cellulose, hemicellulose and lignin of cassava bagasse were analyzed following NREL protocol [8].

2.2.2 Sugar composition

To determine the sugar composition of the samples, the samples were firstly hydrolyzed by sulfuric acid, then released sugar composition was determined by HPLC (Agilent 1200 series, Germany) using HPX-87P column (Bio-Rad, USA) at flow rate 0.6 mL/min; 80 ^oC.

2.2.3. Glucose determination

Glucose concentration of the hydrolysate was determined using D-Glucose Assay Kit GOD-POD Format (Megazyme, USA) following the manufacture introduction. Samples were heated to 95 ^oC for 5 min to deactivate the enzymes in the hydrolysate. Sample (15µl) was added to 150 µl GOD-POD reagent and let for reaction in the dark at 30 ^oC for 30 min. The standard and blank reactions were carried out with 15µl of 1.0 g/L of glucose solution and 15 µl of distilled water, respectively. The absorbance of the reaction mixtures was measured at 510nm (Synergy HT Multi-Mode Microplate Reader, Biotek, USA).

Glucose concentration of the hydrolysate was calculated as:

$$C_{glucose}\,(g/L) = \frac{A_{sample} - A_{blank}}{A_{standard} - A_{blank}}\,xf$$

$C_{glucose}$: Glucose concentration of the hydrolysate (g/L). A_{sample}, $A_{standard}$, A_{blank}: Absorbance of the sample, standard and blank reactions, respectively. f: Dilution factor

2.2.4. Reducing sugar determination

Reducing sugar of the samples was determined using DNS method [9]

2.2.5. Scanning electron microscopy analysis (SEM)

Physical changes in the native and starch extracted bagasse surface were observed by FESEM (JEOL JMS-7600F) at 10 kV.

2.2.6. Glucoamylase activity assay

One GAU is defined as the amount of enzyme that hydrolyzes the starch and releases 1 µmol glucose in 1 minute. Briefly, 300 µl of diluted enzyme was mixed with 600 µL of starch solution (1%) in acetate buffer 0.05 M, pH 4.7. The reaction was carried out at 30 ^oC for 10 minutes, then enzyme was deactivated by heating at 100 ^oC for 2 minutes. Glucose released was determined by GOPOD kit.

2.3. Enzymatic treatment of cassava bagasse

The bagasse was treated with Stargen 002. The amount of enzyme loading changed from 5 to 60 GAU/g of dry cassava bagasse. The reactions were carried out at pH ranging from 3.5 to 5, in 1 L bioreactor with agitation 150 rpm, at different temperature (37-50 ^oC) for 48 h. The solid consistency was changed from 5 to 20 %.

After the reaction, the glucose syrup was separated using filtration. The pulp was washed 3 times by water, then used to determine the remaining starch using Total Starch Kit (Megazyme, USA). The glucose in syrup was determined by GOPOD kit (Megazyme)

The starch hydrolysis yield was expressed as the percentage of glucose released in the syrup comparing to the bagasse starch glucose.

Enzyme reaction rate was calculated as:

$$Rate = (C_t - C_{t-1})/\Delta t \;\; (mg.L^{-1}.h^{-1})$$

Where C_t and C_{t-1} were glucose concentrations (mg/L) of the hydrolysates at hydrolysis time t and t-1, respectively; Δt was duration between t and t-1 (h).

3. Results and discussion

3.1. Cassava bagasse composition

The cassava bagasse contained 55±1.3 % of starch, 25±2.01 % of cellulose and other component as lignin (5±0.45 %) and hemicellulose (3.5±0.5 %). The composition of the bagasse was similar to the previous publication [1, 10]. Observing in light microscope and SEM, it was clearly seen the granular starch entrapped in the lignocellulose fiber matrix (Fig.1). In order to liberate the starch, it is necessary to breakdown the lignocellulose matrix or resize the starch granular.

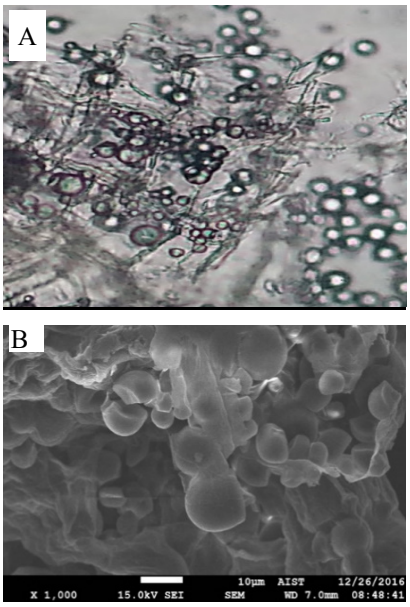

Fig. 1. Image of cassava pulp surface before starch hydrolysis. (A) Light microscope objective 40x, (B) SEM image

3.2 Starch hydrolysis conditions

In the previous reports, entrapped starch was recovered by using cellulase or mechanical treatment to degrade cellulosic matrix of the bagasse was [2, 7]. In this study, Stargen^{TM} 002 was used for recovery of glucose from cassava bagasse. According to the producer, this product contains *Aspergillus kawachi* ∝-amylase expressed in *Trichoderma reesei* and glucoamylase from *Trichoderma reesei* that work synergistically to hydrolyze granular starch to glucose. Both enzymes work at 50^{o}C on non-gelatinized granular starch.

3.2.1 Effect of pH on starch hydrolysis

Enzymatic activity is generally affected by pH's reaction. The optimal pH for Stargen 002 amylase is from 4 to 4.5. To find the favorable pH for the enzyme reaction, the hydrolysis was carried out at different pH from 3 to 5 with interval 0.5. The result was illustrated in Fig.2.

The results in Fig.2 showed that, pH 3 was not favorable for enzyme to work. In the range of pH 3.5-5, at first 9 hours of hydrolysis the average reaction rates were in order of pH 4> pH 3.5~ pH 4.5>pH 5. From 9 to 24 h of hydrolysis, the reaction rates sharply decreased. At 24 h, all rates dropped to similar value of 1.2±0.04 g glucose/L.h (Fig2A). The decrease of the reaction rates can be explained by the decrease of the starch substrate after 24 h of the reaction.

After 48 h of hydrolysis at pH 3, the reducing sugar and glucose released in the hydrolysate were 8.8 and 7.96 g/L, respectively; the starch hydrolysis yield was only 22.27 % (Fig2B). In contrary, the reducing sugar, glucose concentration and starch hydrolysis achieved 35 g/L, 30 g/L and 98 % respectively, in the range of pH from 3.5 to 5. These results suggested once confirmed the pH range for starch hydrolysis using Stargen 002 could be from 3.5 to 5.

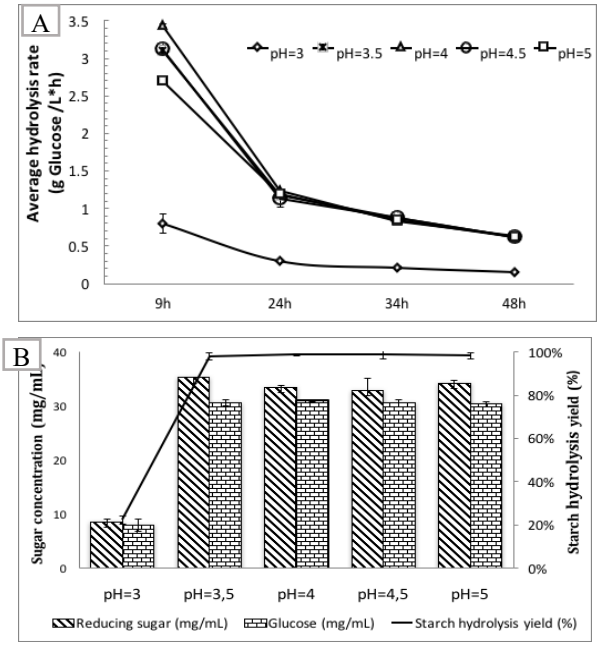

Fig. 2. Influence of pH on the hydrolysis reaction of residue starch in the bagasse using Stargen 002 (5 % solid consistency, 50^{0}C, 48 h): A: reaction rate and B: sugar content in the hydrolysate and hydrolysis yield.

3.2.2 Effect of enzyme loading on starch hydrolysis

In this research, the reactions were performed at pH 4. The amount of enzyme utilized in hydrolysis reaction increased from 5 to 10 GAU/g dry bagasse. The average hydrolysis rates at first 9 hours were proportional to the enzymes loading (Fig.3A). Similarly, to the Fig.2, from 9-24 h of the reaction, the reaction rates also sharply dropped. At 24 hours of hydrolysis, these rates of all reactions were approximately similar.

The reducing sugar, glucose concentration and starch hydrolysis yield after 48h was shown in Fig.3B. Increase of the enzyme loading from 5 to 10 GAU/g dry bagasse resulted on the increase of starch hydrolysis yield from 89.08 % to 97.25%. Further increase of the enzyme loading from 10-30 GAU/g did not improve the hydrolysis yield significantly. Therefore, the enzyme loading of 10 GAU/g was chosen for the next experiments.

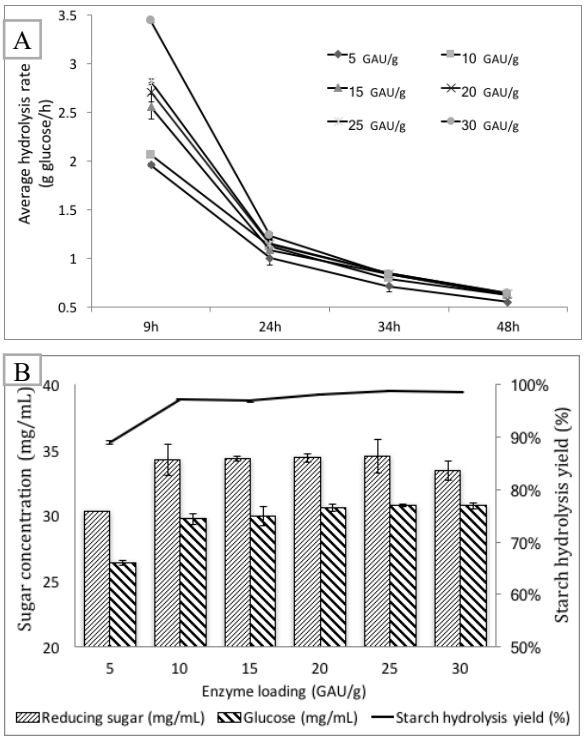

Fig. 3. Influence of enzyme loading on the hydrolysis of residue starch in the bagasse using Stargen 002. A: reaction rate and B: sugar content in the hydrolysate and hydrolysis yield.

3.2.3 Effect of temperature on starch hydrolysis by Stargen 002

In cassava bagasse, starch presents in granule form, which litmit the direct hydrolysis by conventional amylases. To be hydrolyzed, the starch has first passed a gelatinization step. During the heating starch solution (gelatinization), water is firstly absorbed in the amorphous space of starch, leading to swelling the starch. Then water enters via crystalline regions, the tightly bound areas of double helical structure of amylopectin. At ambient temperature, these crystalline regions do not allow water to enter. Heat causes such region to become diffused, the amylose chains begin to dissolve, separating it into amorphous forms, the number and size of crystalline region decrease. Increase the temperature resulted in increase the deroganization. As a result,, the starch becomes more vulnerable againts the enzyme attack. For this reason, in industry, to convert starch to glucose, twostep process is currently carried out which requires energy, equipment and time costs.

In this research, a direct starch hydrolysis was investigated using Stargen 002, DuPont as the enzyme can directly hydrolyze the starch granular without gelatinization step. The optimal temperature for hydrolysis reaction was examined at different temperature ranging from 30˚C to 50 ˚C.

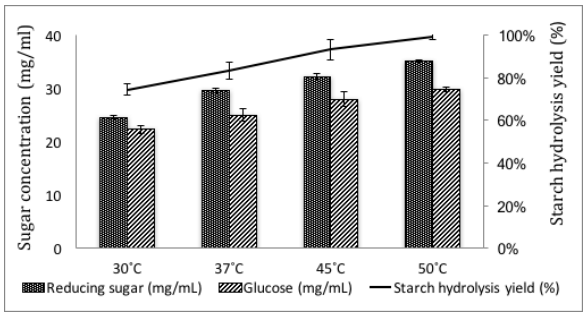

Fig. 4. Effect of temperature to the starch hydrolysis process using Stargen 002, DuPont at 5% solid consistency

The Fig.4 showed the effect of reaction temperature on glucose yield released from cassava bagasse. Within study temperature range, the increasing the reaction temperature improved the reducing sugar, glucose concentration released in the hydrolysate, then ameliorated the starch hydrolysis yield. At the lower temperature ranging from 30 to 37^{o}C, from 74.5 to 82.3 % starch were hydrolyzed after 48 h. This result confirmed the capability of hydrolysis of Stargen 002 against non-cooking starch.

At 50^{0}C, the hydrolysis yield attained the highest value, upto 99.3±1.5% after 48 h, the glucose concentration in hydrolysate accumulated to 29.8±0.44 g/L.

3.2.4 Effect of solid consistency in the hydrolysis reaction on glucose recovering yield

In this study, the solid consistency from 5 to 20 % were experimented. As shown in the Fig.5. The starch hydrolysis yield depended on the bagasse solid loading. The higher solid consistency utilized in hydrolysis reactions, the higher glucose concentration in the hydrolysates was attained. However, higher bagasse consistency in the reaction caused high viscosity and reduced the reaction efficiency due to poor mixing. When bagasse concentration increased from 5% to 10%, the hydrolysis yield remained unchanged, achieved 99.6-99.9%.

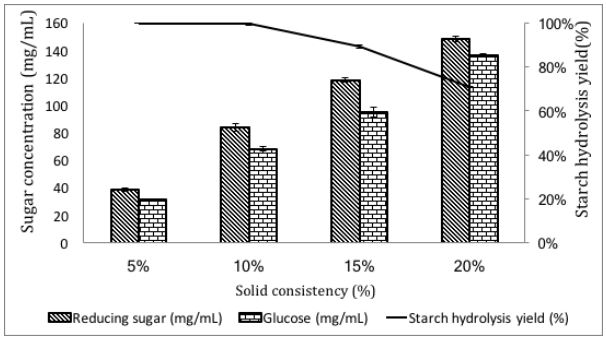

Fig. 5: Effect of solid consistency loaded into the hydrolysis reaction of bagasse starch to the starch hydrolysis yield.

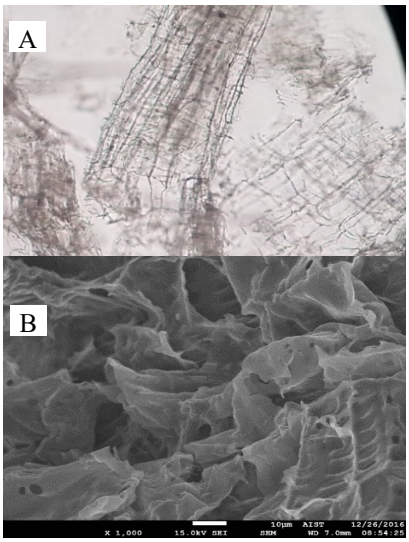

Fig. 6. Image of cassava pulp surface after starch hydrolysis at 10% solid consistency: Light microscope objective 40x (A) and SEM image (B).

The light microscope and SEM images of the bagasse after starch hydrolysis of 10% solid loading reaction confirmed almost no starch granules could be observed (Fig.6).

The reducing sugar and glucose concentration in the hydrolysates increased with the increasing of solid consistency in the reactions. Overall, at 20% solid consistency, the reducing sugar and glucose concentration attained 148 and 137 mg/mL respectively. This glucose solution could be used in plentiful applications and starch-free cellulose could be served for cellulose based materials.

However the hydrolysis yield was dramatically decreased when bagasse content further increased; the starch hydrolysis yield dropped down to 89.23 % and 70.95% at 15 and 20 % solid consistency, respectively. It was due to the improper mixing in the reaction resulting in the inappropriate contact of enzymes to the fiber matrix to cleave the starch. Therefore, the choice of bagasse loading rate may be determined based on the specific application.

4. Conclusion

Glucose can be directly recovered from cassava pulp by using the granular amylase enzyme Stargen 002 in one step process. The pulp can be treated in the reaction of 10%-20% solid content with 10 GAU/g dry bagasse at pH 3,5-5, temperature of 50 ^{o}C for 48 h to recover totally glucose from starch remaining in the cassava bagasse. The obtained glucose syrup of 137 g/L can be obtained with 20% bagasse loading in the hydrolysis and used for fermentation of desired products. The starch-free pulp can be used for cellulose materials development. The process can help to add values to the cassava bagasse and the cassava production chain.

Acknowledgments

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