

## EFFICIENT STARCH RECOVERY FROM CASSAVA BAGASSE: ROLE OF CELLULASE AND PECTINASE

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**Abstract.** Cassava is an important industrial crop in Vietnam for the production of cassava starch as the key raw material in biotechnology and related industries. However, the cassava starch industry discharges a large amount of wet bagasse. The bagasse, which contains 50-60 % starch and 25-30 % cellulose, is currently used with low value and causes severe environmental concerns. In another way, the bagasse can be a source of value-added products if starch is recovered and resulting cellulose is used to develop renewable materials. Recovery of starch and cellulose from the cassava bagasse will have a double impact: improve the total value of cassava production and reduce the risk of environmental pollution. Residue starch granules are entrapped in the cell wall matrix. Therefore, to recover starch from the bagasse and cellulose thereof, the cell wall matrix of bagasse should be cleaved but not be degraded. In this paper, enzymes were used for treating the bagasse, liberating the starch and resulting cellulose materials. The samples of cassava bagasse were treated either with pectinase (Pectinex Ultra SP-L, Novozymes) and cellulase (NS22192, Novozymes) alone or with their mixture at various enzyme ratio. The response surface methodology was used to fit the experimental data and investigate the enzyme interaction and the influence of the enzyme loading on the starch recovery yield. The highest starch yield of 87.85 % was achieved by treatment of the bagasse with 13.5 CMCU and 7 PGU per gram of dry bagasse at 45 °C and pH 4.8 for 4 hours at the solid loading 5 %.

**Keywords:** starch recovery, cassava bagasse, cellulase, pectinase.

**Classification numbers:** 1.3.1, 1.4.2.

### 1. INTRODUCTION

Cassava productivity in Vietnam achieved 10.35 million tone of cassava roots of which 55 % were used for cassava starch production. Starch processing generates a large amount of cassava bagasse (or cassava pulp) as solid waste [1]. It is estimated of 280 tons of bagasse with high moisture (85 %) per every 250-300 tons of cassava roots processed [2]. The high humidity in cassava pulp makes them susceptible to be decay, causing serious environmental concerns. Cassava bagasse is a fibrous residue, which contains more a half of starch (50-60 %), cellulose (20-30 %) and minute amount of hemicellulose and lignin [2]. The bagasse fibrous network holds starch granules in its matrix, therefore, it remains in this complex matrix and presents at

high amount in bagasse waste [3]. Cassava bagasse, without cyanide, is currently used for animal feed or fertilizer with relatively low value added due to its low protein content [2; 4].

To release the starch granule from the cellulose matrix, extraction of starch from cassava bagasse by using multi-enzyme treatment could be a choice. Number of reports suggested the benefit of using such enzyme to treat the cassava bagasse. A treatment of bagasse with a mixture of 15 units of cellulase and 122.5 units of polygalacturonase per gram of bagasse for 60 mins resulted in 40 % starch recovery [3]. Balagopalan used cellulase and pectinase with 0.2; 0.3 and 0.4 % loading, enhanced the starch recovery to 7.1; 15.9 and 37 %, respectively [5]. Those starch yields, however, were considered rather low at high enzyme rates used. In addition, the effect of enzymes on cellulose was not investigated. Physical treatment was another solution additionally used to treat the cassava bagasse. Severe disruption of cellulose network was observed after 40 s of sonication and starch recovery was estimated approximately of 15.69 % [3]. Recovering bagasse starch at higher rate and use the resulted cellulose is somehow remained challenging which is attractive further investigation.

Enhancing the starch recovery rate from cassava pulp in minimizing the enzyme dose and reducing the loss of cellulose content is objective of our research efforts. Our preliminary study suggested much lower enzyme activities, in particular pectinase, could be loaded in the reaction to gain a higher starch extract rate. In this research, using selected enzyme mixture, attempt was made to enhance the starch recovery rate from cassava pulp collected from Yen Bai Cassava Starch Company. The interaction of enzymes in the extraction of residual starch was investigated. The enzyme loading rate for starch extraction was also examined and discussed.

## 2. MATERIALS AND METHOD

### 2.1. Materials

Fresh cassava bagasse was collected from Van Yen Cassava Factory, stored at -20 °C for further use.

The enzymes used in the study were provided by Novozymes (Denmark) including: NS22192, 2050 CMCU/mL and Pectinex Ultra SP-L, 1856 PGU/mL.

### 2.2. Analysis

#### 2.2.1. Composition of cassava bagasse

The starch contained in cassava bagasse was determined using the Total starch kit of Megazyme (USA). Briefly, cassava bagasse was ground to pass 0.5 mm screen. 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 hydrolyzed using amylase ( $\alpha$ -amylase, glucoamylase), the glucose released was measured and convert to starch content by multiple with 1.1 coefficient.

Cellulose, hemicellulose and lignin of cassava bagasse were analyzed following NREL protocol [6]. The free-lignin residues were firstly hydrolyzed by sulfuric acid, then released sugar composition was determined by HPLC (Agilent 1200 series, Germany) using Aminex HPX-87P column (Biorad, USA) with sample volume of 20  $\mu$ L. Mobile phase was purified water (Merck, Germany) with flow rate of 0.6 mL/min. The analysis was run at 80 °C with detector RID and retention time of 25 min.

### 2.2.2. Glucose determination

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

Glucose concentration of the hydrolysate was calculated as

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

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

### 2.2.3. Reducing sugar determination

Reducing sugar of the samples was determined using DNS method [7].

### 2.2.4. Scanning electron microscopy analysis

Physical changes in the surface of native and starch extracted bagasse were observed by FESEM (JEOL JMS-7600F) at 15 kV.

## 2.3. Experiment design

### 2.3.1. Enzymatic treatment of cassava bagasse

The bagasse was treated with cellulase (15 CMCU/g dry mass) and pectinase (15 PGU and 122.5 PGU/g dry mass) singly or in combination in 0.05 M citrate buffer, pH 4.8 at the solid consistency of 5 % dry mass. The treatment was carried out in shaking incubator at 120 rpm, 50 °C for 4 h. The reaction was stopped by heating to 100 °C during 2 min. Then, the mixture was filtered and washed twice with water. The filtrate was passed through a 90-mesh sieve and decanted after keeping overnight at 4 °C. The water was carefully drawn off, the solid was dried at 55 °C until dry. The starch recovery efficiency was expressed as the percentage of liberated starch to total residue starch.

### 2.3.2. Response surface methodology (RSM) designed

The central composite matrix experiment was designed to investigate the effect of study enzymes and the optimal ratio between cellulase and pectinase for starch recovery. In this study, a RSM combined with Box-Wilson design was used to study. The design was composed of three levels (low, medium and high, being coded as -1, 0 and +1) and a total of 11 runs were carried out to optimize the level of two independent variables: pectinase (A) and cellulase (B) loading rates. According to our preliminary experiments, cellulase and pectinase were loaded from 3 to 15 CMCU/ g and from 7 to 83 PGU/ g dry substrate, respectively. The range and levels used in the experiments were listed in Table 1. The results were analyzed by applying the coefficient of

determination ( $R^2$ ), response plots and analysis of variance (ANOVA) using Design Expert 11 software.

Table 1. Independent factors and corresponding levels.

Variables	Real values of coded levels		
	-1	0	1
Pectinase (PGU/g dm)	7	45	83
Cellulase (CMCU/g dm)	3	9	15

### 3. RESULTS AND DISCUSSION

#### 3.1. Cassava bagasse composition

Cassava bagasse was collected from Van Yen Cassava Factory (Yen Bai Joint Stock Forest Agricultural Products And Foodstuff Company). The composition of this material was shown in Fig. 1A.

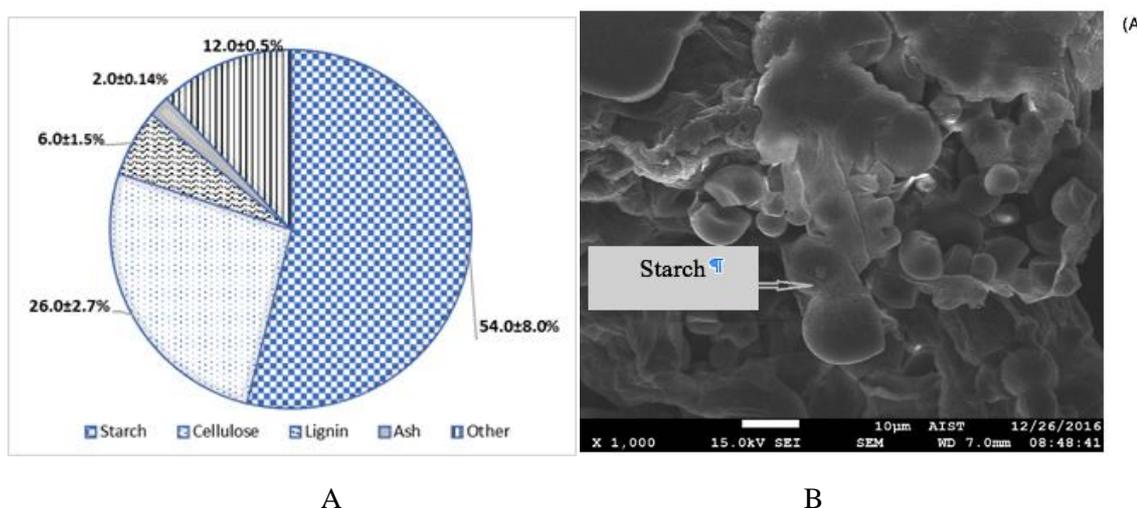


Figure 1. Cassava bagasse composition (A) and SEM image of cassava bagasse surface (B).

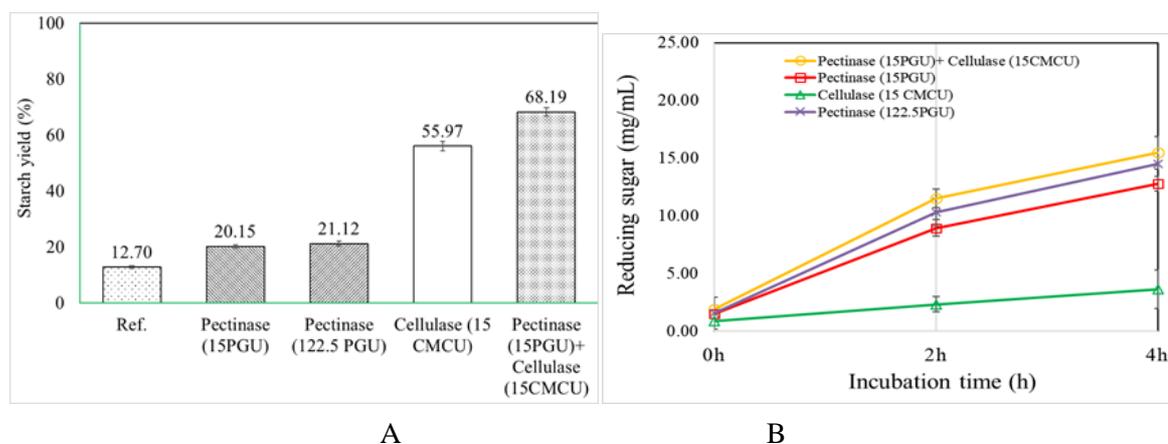
According to Fig 1A, starch was the main component of cassava bagasse ( $54.0 \pm 8.0$  %), followed by cellulose ( $26.0 \pm 2.7$  %). The composition of Vietnam cassava bagasse was similar to the other countries' bagasse [2, 8]. In the bagasse, the starch exists in free or trapped form in lignocellulose fiber matrix due to the big size of starch granules (Fig. 1B). The free starch could be recovered during washing step; however, the trapped form may be exclusively liberated only if the lignocellulose network is cleaved. After the removal of starch, the residue became a cleaner cellulose source thank to the low lignin content in the materials.

#### 3.2. Selection of enzymes to recover bagasse starch

To release the entrapped starch granule, the fiber matrix must be broken down. For further use of cellulose, the cellulose fiber is needed to be fragmented only and the cellulose loss due to

hydrolysis should be controlled. The main enzyme can be used to liberate embedded starch from fiber bagasse is thus endo-glucanase, which break down the cellulose network by cutting randomly the endo- $\beta$ -1,4-glycosidic linkages, cleaving the cellulose fiber, but not hydrolyzing it to glucose. Based on the primary selection of the enzymes (data not shown), among cellulases, NS22192, a cellulase from Novozymes, having appropriate endoglucanase/exoglucanase ratio for starch recovery will be used in this research. Pectin is also present in the fiber network. Similarly, the pectinase with polygalacturonase activity of Pectinex Ultra SP-L, Novozymes will be chosen for cleavage the pectin in the matrix. To understand the role of these enzymes in the treatment, the bagasse was treated with either single cellulase, pectinase or mixture of these two enzymes. The sample without enzyme treatment (Ref.) was used as the reference. The results were shown in Fig. 2.

The reference sample without enzymatic treatment gave a starch recovery of 12.7 %. It corresponded to the amount of free starch which was washable from cassava bagasse. In all three treatments with enzymes, the starch yields were higher than that of the reference sample. The enzymes broke down polysaccharide chains in cassava bagasse (shown by the high reducing sugars released), liberating the starch. Both pectinase treatments with 15PGU and 122.5 PGU/g gave a high amount of reducing sugar (Fig. 2B), resulting in an increase of the starch removal yield compared to that of the reference sample. This result is the evidence for the effect of pectinase on the matrix network to release the starch, confirming the role of pectinase in the treatment of the bagasse. However, the starch removal in these two pectinase treatments yielded similarly, 20.15 % with 15 PGU/g and only 21.12 % with very high Pectinex loading. It may be due to the limit amount of pectin in the cassava bagasse (appx. 7 %) therefore the pectinase dose for the treatment should be optimized.



*Figure 2.* Role of enzymes on starch recovery yield (A); and reducing sugar released from cassava bagasse (B). The cassava bagasse (5 %) was treated with cellulase (15 CMCU /g dm) and pectinase (15 PGU/g dm). The reaction mixture was incubated for 6h at 50 °C and pH 4.8.

Between the two enzymes used, pectinase had limited effect (20.15 %), while cellulase played a more significant role in the release of starch (starch removal yield achieved 55.97 %). On the other hand, the reducing sugar obtained by treatment with cellulase NS22192 was rather low but the starch yield was much higher than with pectinase treatment. This showed that cellulose had been mainly fragmented but not saccharified. The observation confirmed both the need for cellulase treatment and the appropriate selection of cellulase enzyme for bagasse treatment to efficiently recover both starch and cellulose for further use. By the combination of

two enzymes for the treatment, the starch recovery was enhanced up to 68.19 %, which was 1.2 and 3.38 folds higher than those obtained by the single use of cellulase or pectinase, respectively. The reducing sugars released in this case were corresponding to the sum of reducing sugars released from both reactions with single enzymes. That observation proved that the cleavage of polysaccharide chains by enzyme mixture was similar to the treatments by single enzyme each, no further cellulose degradation was observed. The synergetic effect of cleavage of both fibrous chains by two enzymes led to increase the starch removal due to both chains opened.

### 3.3. Effect of enzyme ratio on the starch recovery

Table 2. Experimental matrix and yield of starch recovery.

N <sup>0</sup>	Pectinase (PGU/g)	Cellulase (CMCU/g)	A	B	Starch recovery (experiment) (%)	Starch recovery (predicted) (%)
1	83	9	1	0	53.04	52.69
2	7	3	-1	-1	48.34	49.69
3	83	3	1	-1	51.13	52.47
4	45	9	0	0	63.53	63.28
5	45	3	0	-1	53.37	54.01
6	45	15	0	1	71.90	72.55
7	7	9	-1	0	81.67	81.21
8	83	15	1	1	76.14	77.49
9	7	15	-1	1	86.55	87.91
10	45	9	0	0	62	63.28
11	45	9	0	0	63.37	63.28

To examine the effect of the ratio between 2 enzymes for the treatment, a matrix of experiments in 1L reactor were carried out as shown in Table 2. In these experiments, cellulase loading varied from 7 to 83 CMCU/ g, while pectinase was loaded from 3 to 15 PGU/ g dry substrate. The obtained data were analyzed using Design Expert software.

The linear and quadratic models were first suggested by the Design Expert 11 software to fit the experimental data. The ANOVA analysis showed that both *p*-values of these models were less than 0.05 (0.0017 and 0.0453, respectively). Two models were considered significant. However, the *p*-values of lack of Fit of both models (0.0124 and 0.008) indicated that both lack of Fit were significant, both models thus failed to explain the data variations.

A reduced cubic model by adding the interaction  $A^{2*B}$  and  $A*B^2$  was used [9, 10, 11]. ANOVA analysis showed the model was significant and the lack of Fit was not significant (Table 3). The reduced cubic model therefore can be used to fit the experimental data.

The dependence of the starch recovery yield on pectinase and cellulase loading was expressed as in the following equation (insignificant coefficients were removed):

$$\text{Starch recovery (\%)} = 63.28 - 14.32*A + 9.27*B - 3.3*A*B + 3.61*A^2 + 6.54*A^2*B + 12.41*A*B^2.$$

The  $R^2$  and adjusted  $R^2$  were 0.998 and 0.994, respectively. These values indicated the close agreement between experimental data and the predicted values. The response surface model displaying the dependence of starch recovery yield on the enzymes loading was shown in Figure 3. The response surface plots showed that the higher the cellulase rate, the higher the starch recovery. However, the effect of pectinase was complicated, and it depended also on the cellulase concentration.

Table 3. ANOVA analysis for reduced cubic model.

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	1671.82	7	238.83	255.52	0.0004	significant
A-Pectinase	409.84	1	409.84	438.47	0.0002	
B-Cellulase	171.68	1	171.68	183.67	0.0009	
AB	43.56	1	43.56	46.60	0.0064	
A <sup>2</sup>	32.97	1	32.97	35.27	0.0095	
B <sup>2</sup>	3.13	1	3.13	3.35	0.1645	
A <sup>2</sup> B	57.03	1	57.03	61.01	0.0044	
AB <sup>2</sup>	205.34	1	205.34	219.69	0.0007	
Residual	2.80	3	0.9347			
Lack of Fit	1.39	1	1.39	1.96	0.2960	Not significant

The optimal condition for starch recovery was calculated by Design Expert software using the model. Totally, 35 solutions for the starch recovery of more than 87 % were suggested. The highest starch yield attained up to 88.56 % at enzyme loading rate of 7 PGU/g and 13.5 CMCU/g. The experimental data later at the optimal enzyme ratio confirmed this calculation with the recovery yield achieved 87.85 %, closely to the predicted value.

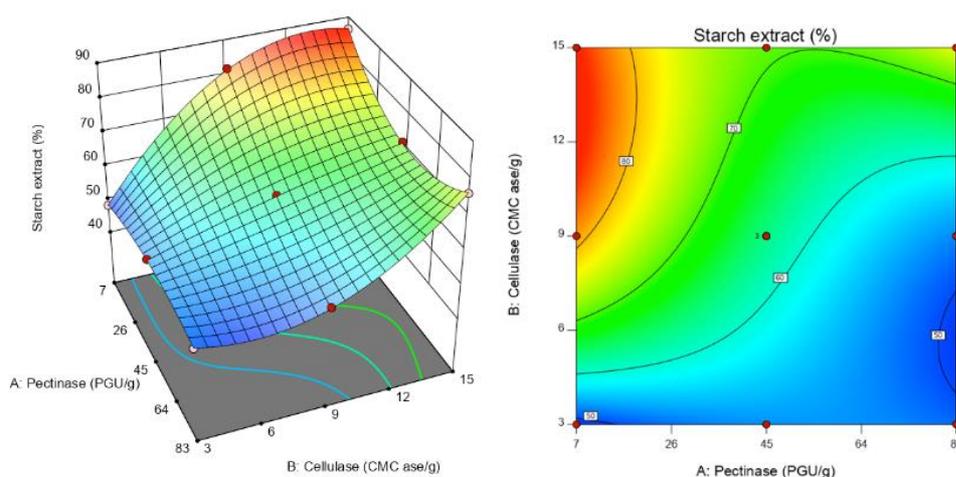


Figure 3. Response surface plots, showing the effect of cellulase and pectinase loading to starch recovery.

This result is quite higher than that of other treatments of bagasse reported. The treatment with similar cellulase dose of 15 units cellulase and a very high polygalacturonase dose (122.5 units polygalacturonase/g) to obtain only 40 % starch recovery yield [3]. Kallabinski and Balagopalan also used NS22192 and Pectinex SP to extract starch from cassava roots and achieved a maximum starch yield of only 26.08 % [12]. Based on the RSM result, it may be suggested that the lower efficiency of starch extraction in that research may be due to the very high pectinase used in the report. In their study, the pectinase rate was similar to that in this study, but cellulase loading was lower than 30 %. In addition, the enzymes were used to extract the starch from the roots, it could be a cause of an inhibition of the enzyme action due to starch abundant in the samples.

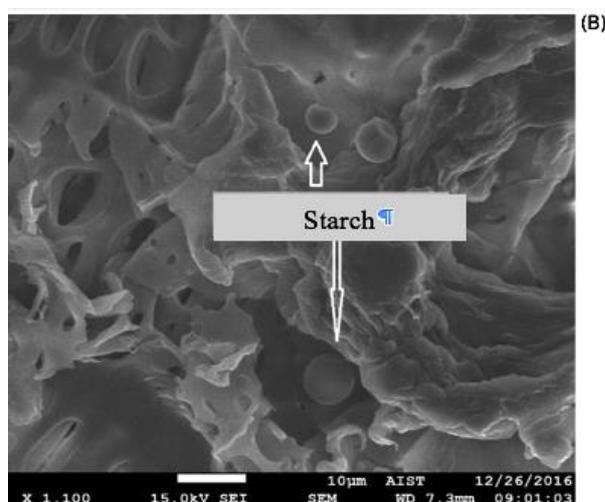


Figure 4. SEM image of cassava pulp surface after starch recovery.

The SEM images of cassava bagasse after starch recovery were shown in Fig. 4. Compared to the image of initial cassava bagasse this image showed that a large number of starch granules were kept in the cellulose networks (Fig. 1B), only few starch granules could be found in the deeper region of the bagasse cellulose network after the treatment, when 86.55 % starch had been removed. To remove this minute amount of starch, it may need a further degradation of cellulose network.

Regarding the influence of enzymes on the starch yield, it can be seen that the more cellulase is used in the treatment, the higher starch yield can be achieved. But in the reality, from economic point of view, we also need to consider the appropriate enzyme dose to be used in the treatment.

#### 4. CONCLUSION

Cellulase and/or pectinase can disrupt the fibrous structure of cassava bagasse, allowing embedded starch granules to be extracted. Treatment of the bagasse with cellulase had a stronger effect on the bagasse network than that with the pectinase. The combination of both cellulase and pectinase for bagasse treatment gave a more efficient starch recovery than individual enzyme use.

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