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A Review of Bioethanol Production from Cassava Peel (*Manihot esculenta*)

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Abstract:
***Background**: In Indonesia, cassava plants are found in almost throughout the archipelago and are used as staple food. However, the utilization of cassava peel is still not optimal. Cassava peels are widely used as fertilizer and livestock feed, or are just treated as waste and thrown away. In recent years, global energy demand has been increasing, in line with population and economic growth. Therefore, it is very important to find safe, easy to obtain and renewable energy source. In the last decade, many studies have been conducted on the use of cassava peel as a safe and effective source of renewable energy. This review article aims to provide an overview of the production of bioethanol from cassava peels through the hydrolysis process by yeast (Saccharomyces cerevisiae).*
***Materials and Methods**: Literature review was used to find information from international journals in the last ten years (2012-2022) through online media using the keywords bioethanol and cassava peel. This review article is sourced the information from sciencedirect, google scholar, research gate, and others.*
***Results**: Based on a review of several articles, it can be concluded that cassava peel (Manihot utilissima) produced bioethanol through hydrolysis (using HCl, H_2SO_4, SO_3H, and Aspergillus niger) and fermentation (using Saccharomyces cerevisiae).*
***Conclusion:** In general, cassava peel has not been utilized properly. Bioethanol can be produced from cassava peels. Bioethanol derived from cassava peels can be used as an alternative to renewable natural resources. Production of bioethanol from cassava peel only requires cheap and obtainable materials. This has the potential to become a source of wealth for tropical countries if utilized optimally.*
***Keywords**: bioethanol; cassava; hydrolysis; fermentation*

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I. Introduction

Cassava (*Manihot esculenta* Crantz) is a plant from the Euphorbiaceae family and is classified as a tropical plant [1]. In 2015, Indonesia has reached 22 million tons for cassava production. However, a lot of cassava peel wastes produced from cassava processing have not been used optimally. Cassava peel mostly used for animal feed or just thrown away [2]. Several studies have stated that cassava peel can be used as an energy source in the form of ethanol. The need for ethanol as a solvent, disinfectant, raw material for chemical factories, and as an alternative energy substitute for fuel oil (petrol) is increasing [3]. The energy needs of a country tend to increase along with the economic growth. In general, the world's energy needs are still depend on fossil resources, especially oil, gas, and coal. These natural resources have been formed since thousands of years ago [4].

One kilogram of cassava can produce 10-15% of peels waste. These peels are consist of 37.9% cellulose; 37% hemicellulose; and 7.5% lignin, which is suitable as raw material for bioethanol production [5,6]. Bioethanol is a renewable resources, meaning that even it is taken continuously, its availability in nature will not run out. This bioenergy also produces less pollution and can be produced from inexpensive materials containing sugar and starches such as corn, potato peels, wheat bran, bagasse, and molasses [7].

Bioethanol is obtained from the process of converting simple sugars into ethanol and carbon dioxide (CO_2) [8]. There are two main stages in the production of bioethanol. The first process is hydrolysis which breaks down cellulose into simple sugars with the help of acids or enzymes. The second process is fermentation which will convert sugar into alcohol through anaerobic respiration by microbes [9]. *Saccharomyces cerevisiae, Aspergillus niger* and mucromucedo are commonly used for the production of first generation bioethanol [10]. The addition of yeast in the process provides the required enzymes (invertase and zymase) in the production of bioethanol. The fermentation process can be represented by the chemical equation shown [11].

$$C_{12}H_{22}O_{11} + H_2O \xrightleftharpoons{\text{Invertase}} C_6H_{12}O_6 + C_6H_{12}O_6$$

Sucrose Water Fructose Glucose

$$C_6H_{12}0_6 \xrightleftharpoons{\text{Zymase}} 2C_2H_5OH + 2C0_2\ (\text{gas})$$

Fig 1. Chemical Reaction of Sucrose Fermentation into Ethanol with the Help of Enzymes [11]

Cassava peels that is treated as wastes can actually be processed into useful products that are benefiial to the community. Cassava peels contains high carbohydrates, so it is very suitable as the material to produce bioethanol. Therefore, it is necessary to conduct research on the manufacture of bioethanol from cassava peel [12].

II. Material And Methods

Cassava peels (*Manihot esculenta*) were collected and washed with water. Then washed, cut into small pieces and dried using oven. The dried cassava peel was then crushed using a machine. The next step was hydrolysis and fermentation. Distillation was then carried out to obtain the final product in the form of bioethanol.

This article was compiled through a literature review. The information sourced from international journals in the last ten years (2012-2022) through online media was searched using the keywords bioethanol and cassava peel. The literatures used was obtained from sites such as science direct, google scholar, research gate, and others.

III. Results

From a review conducted in ten journals over the last ten years by comparing the bioethanol production methods, a summary can be drawn as shown in the table below.

Table 1. Comparison of several reviews in producing bioethanol

No.	Titles/ Journals	Author	Methods		Yields
			Hydrolysis	Fermentation	
1	Bio-Ethanol Production from Non-Food Parts of Cassava (*Manihot esculenta Crantz*). AMBIO, 2012 [13]	Ephraim Nuwamanya, Linley Chiwona-Karltun, Robert S. Kawuki, Yona Baguma	HCl, NaOH, enzymatic	*Saccharomyces cerevisiae*	60,2%
2	Bioethanol production from cassava peels using different microbial inoculants. African Journal of Biotechnology, 2016 [14]	Obianwa Chibuzor, Edak A. Uyoh, Godwin Igile	-	*Aspergillus niger + Spirogyra Africana + Saccharomyces cerevisiae*	41%
3	Bio-Ethanol Production from Cassava (*Manihot Esculenta*) Waste Peels Using Acid Hydrolysis and Fermentation Process. Science World Journal, 2019 [15]	Mustafa Hauwa M., Salihu Dahiru, Bashir Abdulrahman, Ibrahim Abdullahi	H_2SO_4	*Aspergillus niger + Saccharomyces cerevisiae*	37,35%
4	Production of Bioethanol from Hybrid Cassava Pulp and Peel using Microbial and Acid Hydrolysis. Bioresources [16]	Vincent E. Efeovbokhan, Louis Egwari, Edith E. Alagbe, James T. Adeyemi, Olugbenga S. Taiwo	HCl	*Saccharomyces cerevisiae*	33,14%
5	Bioethanol Production from Cassava Peel Treated with Sulfonated Carbon Catalyzed Hydrolysis. Journal of Scientific and Applied Chemistry [17]	Primata Mardina, Chairul Irawan, Meilana Dharma Putra, Sylvera Bella Priscillaa, Misnawatia, Iryanti Fatyasari Nata	SO_3H	*Saccharomyces cerevisiae*	27,72%
6	Production of Bio-Ethanol via Hydrolysis and Fermentation Using Cassava Peel and Used Newspaper as Raw Materials. Materials Science Forum, 2020 [18]	Tintin Mutiara, Siska Widiawati, Syafira Rachmatyah, Achmad Chafidz	HCl	*Saccharomyces cerevisiae*	8,89%

7	Bioethanol production from cassava-based industrial wastesusingacidhydrolysisandsimplefermentation. Journal of Physics: Conference Series, 2020 [19]	A. P. Heriyanti, F. Fibriana, N. L. Tirtasari	HCl	*Saccharomyces cerevisiae*	6,2%
8	Bioethanol Production from Cassava (*Manihot esculenta*) Peel Using Yeast Isolated from Durian (*Durio zhibetinus*). Journal of Physics: Conference Series, 2016 [20]	Hermansyah,Tounaly Xayasene, anNguyen Huu Tho, Miksusanti, Fatma, AlmunadiT.Panagan	H_2SO_4	Ragi yang diisolasi dari buah durian (*Durio zhibetinus*)	1,63%
9	Bioethanol Production by Utilizing Cassava Peels Waste Through Enzymatic and Microbiological Hydrolysis. Earth and Environmental Science, 2017 [21]	R. G. Witantri, T. Purwoko, Sunarto, E. Mahajoeno	Multienzyme treatment, EM4, cellulose	*Saccharomyces cerevisiae*	3,79%
10	Production of Bio-ethanol from Cassava Peels. ResearchGate, 2015 [22]	Olayide R. Adetunji, Pritlove K. Youdeowei, Olalekan O. Kolawole	*Aspergillus niger*	*Saccharomyces cerevisiae*	8,5%

IV. Discussion

The washed cassava peels were cut, dried, and crushed as shown in Figure 2. Then several treatments were carried out by several authors.

Fig 2. **Cassava peels samples were prepared by (A) washing, (B) cutting, (C) drying, and (D) grinding processes** [20]

According to Nuwamanya *et al*. (2012), 200 grams sample was hydrolyzed using 200 ml acid (HCl), 200 ml alkali (NaOH), and 200 ml of a combination of enzymes; amyloglycosidase (60 IU ml^{-1}, SIGMA, Aldrich), amylase (3000 IU ml^{-1}, BDH Laboratories), and cellulases (75 IU ml^{-1}, SIGMA, Aldric) [13].

The comparison of the hydrolysis method showed different amounts of produced sugar. The enzymatic hydrolysis process produced low reducing sugars. This is because enzymes are specific for certain substrates, thus they can affect the fermentation process.

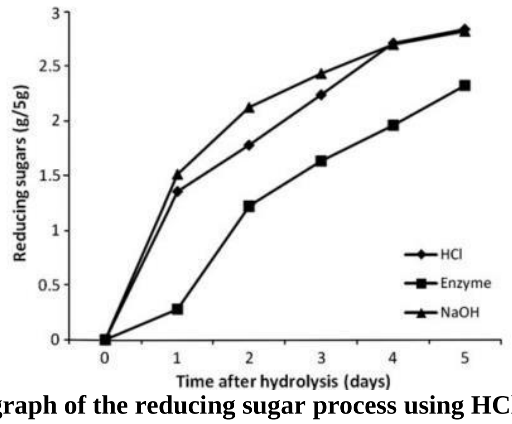

Fig 3. **Comparative graph of the reducing sugar process using HCl, enzymes, and NaOH** [13]

Furthermore, the glucose fermentation process was carried out using the microorganism *Saccharomyces cerevisiae*. The fermentation process took place at room temperature (27^{o}C) at pH 4.5-5. Then, the fermentation results were left for 5 days to observe any increase of the reducing sugar [13]. Analysis of the differences in ethanol levels produced from several parts of the cassava plant can be seen in table 2.

Table 2. Differences in ethanol levels produced from several parts of the cassava plant [13]

Plant part	% EtOH	EtOH (ml)
Roots	61,4	55,8
Skins	60,2	43,5
Stems	59,8	52,4
Leaves	59,5	11,3

The table shows that after five days of fermentation the bioethanol produced from roots (61.4%) and skins (60.2%) was higher than the bioethanol produced from stems (59.8%) and leaves (59.5 %). In the study by Chibuzor *et al*. (2016) 50 grams of cassava peels powder were dissolved in 500 ml of water in several containers. Each container contained 1. 5 ml of *A. niger* + 2 g of *S. cerevisae*; 2. 5 ml *R. nigricans* + 2 g *S. cerevisae*; 3.5 ml *A. niger* + 5 ml *R. nigricans* + 2 *g S. cerevisae*; 4. 5 ml *A. niger* + 1 g *S. Africana* + 2 g *S. cerevisae*; 5.5ml *R.nigricans*+1g *S.Africana*+2g *S.cerevisae*; 6.2g of *S.cerevisae*: and control. For each treatment, 3 repetitions were carried out [14].

Table 3. Percentage of ethanol concentration (g/cm3) from cassava peels on different inoculants [14]

Inoculum	TME 419	TME 0505	TME 4779
A	8.57	10.18	9.37
B	8,57	9.37	10.71
C	8.84	11.00	11.51
D	12.59	13.33	14.46
E	10.98	13.00	10.71
Control	6.43	4.82	7.77

Description: A = *R. nigricans + S. cerevisae*; B = *A. niger + S. cerevisae*; C = *R. nigricans + A. niger + S. cerevisae*; D = *R. nigricans + S. Africana + S. cerevisae*; E= *A. niger + S. Africana + S. cerevisae*; control = *S. cerevisae*

From the results of the study, it can be concluded that *R. nigricans, S. africana* and *S. cereviceae* are the best combination for the production of bioethanol from cassava peels with an optimum yield of 14.46.
Mustafa *et al*. (2019), conducted the production of bioethanol using sulfuric acid in the hydrolysis process with various concentrations. *Aspergillus niger* and *Saccharomyces cerevisiae* were used in the fermentation at 28^{o}C for 4 days [15]. The ethanol yields obtained are shown in table 4.

Table 4. Comparison of the ethanol and sugar concentration produced [15]

No.	Sample	Quantity of ethanol (g/ml)	Sugar level (%)
1	20 g sample + distilled H2O	20.40	4.0
2	20 g sample + 2% H2SO4	23.45	8.3
3	20 g sample + 6% H2SO4	29.80	11.6
4	20 g sample + 10% H2SO4	37.35	15.5

Table 4 shows that hydrolysis using 10% H_2SO_4 produces larger reducing sugar, thus the ethanol concentration obtained is also higher (37.35 g/ml). Efeovbokhan *et al*. (2019) the cassava peel was hydrolyzed with 1 ml of *A. niger* spore suspension and 100 ml HCl in various concentrations. Furthermore, the fermentation using *Saccharomyces cerevisiae* was conducted for 14 days. The results can be seen in Table 5 [16].

Table 5. Ethanol obtained from cassava peels hydrolyzed with HCl at various concentrations [16]

HCl Concentration (M)	Hydrolysis duration (minutes)	Volume of ethanol yield (mL)	Ethanol yield from 20 grams of cassava peels (%)
0.3	20	2.3	9.07
	30	3.4	13.41
	40	4.6	18.15
	50	4.5	17.75
	60	3.8	14.99
	75	4.2	16.57
	90	3.2	12.62
	20	1.3	5.13
	30	2.5	9.86
	40	4.6	18.15

0.5	50	3.6	14.20
	60	5.8	22.28
	75	3.6	14.20
	90	3.2	12.62
0.7	20	2.3	9.074
	30	2.8	11.05
	40	4.3	16.96
	50	6.7	26.43
	60	8.4	33.14
	75	3.6	14.20
	90	3.5	13.81

Table 6. Result of ethanol produced from samples hydrolyzed using *A. niger* [16]

Sample	Ethanol yield (mL)	Weight of ethanol yield (g)	Ethanol yield from 20 gram samples (%)
Cassava peels	5.2	4.1	20.51

The hydrolysis process using 0.3 M HCl for 40 minutes produced the highest percentage of ethanol concentration (18.15%). Meanwhile, at 0.5 M HCl for 60 minutes the resulting alcohol concentration was 22.88%. The maximum alcohol concentration was 33.14% and was obtained using 0.7 M HCl for60 minutes. The process of hydrolysis of 20 grams cassava peels using *A. niger* resulted in an alcohol concentration of 20.51%. Mutiara *et al*. (2019) was hydrolyzed using 50 ml of 0.1 N HCl solution. The fermentation process was performed using *Saccharomyces cerevisiae* for 7 days (18). The results obtained are shown in Table 7.

Table 7. Ethanol production from different material composition [18]

Fermentation time (day)	Main ingredient composition (mL)		Bioethanol concentration (%)
	Cassava peels	Old newspaper	
7	80	20	0
7	60	40	6.194
7	50	50	8.421
7	40	60	8.887

The combination of 80 ml of cassava peels + 20 ml of newspaper solution was resulted in an undetectable alcohol concentration (0%). The concentration of ethanol produced from the combination of 60 ml of cassava peel + 40 ml of newspaper solution was 6.194%. Volume variations of 50 ml of cassava peel + 50 ml of newspaper solution resulted in an ethanol concentration of 8.421%. The concentration of ethanol produced from 40 ml of cassava peel + 60 ml of newspaper solution was 8.887%.

Data analysis showed that the newspaper solution had an effect on the yield of bioethanol concentration. Comparison of the concentration bioethanol produced from different fermentation times can be seen in Table 8.

Table 8. Ethanol production from different fermentation times [18]

Fermentation times (day)	Main ingredient composition (mL)		Bioethanol concentration (%)
	Cassava peels	Old newspaper	
3	50	50	6.690
7	50	50	8.765
10	50	50	9.472

From the table it can be concluded that the longer the fermentation process will have an impact on the yield of bioethanol concentration. The largest concentration of bioethanol (9.472%) was produced on the 10th day of fermentation. The hydrolysis process greatly affects the production of bioethanol. Research by Heriyanti *et al*. (2019) compared the hydrolysis process using various concentrations of 30 ml HCl and H_2SO_4. The fermentation was carried out using *Saccharomyces cerevisiae* at 30^{o}C for several days and the results are shown in the Table 9 [19].

Table 9. Concentration of glucose produced in the hydrolysis process using H_2SO_4 and HCl [19]

Acid	Concentration (% v/v)	Glucose concentration (%)
H_2SO_4	15	4.8
	7	6.0
HCl	15	8.7
	7	0.6

In the hydrolysis process, the highest glucose content was produced with the use of 15% HCl. H^+ in HCl formed free radicals in cassava peels and "pile" powder, reacting with OH^- from water to produce glucose. The use of 7% HCl did not produce sufficient free radicals, therefore the glucose concentration produced was low. Both 15% and 7% H_2SO_4 resulted low levels of acidity, which reduces the water concentration and leads to low glucose levels. Then, fermented with *Saccharomyces cerevisiae* which is able to produce zymase and invertase enzymes to convert glucose into bioethanol [19]. In this study [19], the use of 15% HCl concentration caused the hydrolysis process to produce maximum glucose concentrations, as shown in the graph in Figure 4.

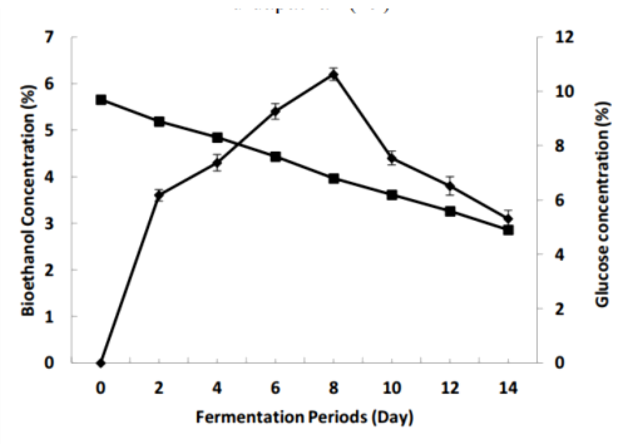

Fig 4. **Graph of relationship fermentation time, glucose concentration, and ethanol concentration produced [19]**

The ethanol concentration increased on the 2nd to the 8th day of fermentation. On the 8th day, the optimum ethanol concentration (6.2%) and glucose concentration (6.8%) was achieved. Yeast has an important role in the fermentation process to convert glucose into bioethanol. In this process, *Saccharomyces cerevisiae* is used as a fermentation agent. Hermansyah *et al*. (2018), conducted a study on the production of bioethanol from cassava peels using H_2SO_4 in the hydrolysis process and yeast isolated from durian fruit in the fermentation process [20]. The yeast was then grown on agar media. Yeast growth results showed the morphological characteristics of small/large, round, shiny, smooth and yellowish as shown in Figure 5.

Fig 5. **Yeast colonies isolate [20]**

From this study [20], the optimum hydrolysis process time was 45 minutes, where the concentration of glucose produced was 11.189%. The concentration of bioethanol produced from fermentation process was 1.63%.

Research conducted by Mardina *et al*. (2021) aimed to determine the potential of cassava peels in producing bioethanol through two main processes, which are using acid in the hydrolysis and fermentation processes. The process of hydrolysis of cassava peels using acid at a temperature of 100°C within 60 minutes with the addition of a carbon sulfonate as a catalyst resulted in 13.53 g/L glucose. The glucose obtained in the hydrolysis process was then fermented at a temperature of 30°C using *Saccharomyces cerevisiae* at pH 4.5 for 24 hours and resulted in 3.75 g/L bioethanol [17].

Adetunji [20] studied the production of bioethanol from hydrolysis of cassava peels using *Aspergillus niger*. *Saccharomyces cerevisiae* was used for fermentation and a distillation process was carried out to obtain the ethanol content. As much as 8.5% ethanol was obtained from 20 grams of the sample which was hydrolyzed at pH 6.71 and 24^0C. The results showed that the starch in cassava peels could be easily degraded by *Aspergillus niger.* The resulting bioethanol has a quality comparable to ethanol in general [22].

Witantri *et al*. (2016) carried out three treatments on the hydrolysis process in the production of bioethanol from cassava peels. The three treatments were the administration of three enzymes, which are: cellulose, multienzyme, EM4, and no-enzymes addition as control [21]. Multienzyme and EM4 are commercially known as livestock supplements. The addition of multienzyme and microbial EM4 can improve digestibility, especially for high-cellulose foods to optimize nutrient absorption [23].

Based on the composition of cassava peels as lignocellulosic biomass, the multienzyme contains enzyme suitable for the hydrolysis processes such as Galactosidase, xylanase, Galactomannanase, β-glucosidase and amylase. The galactosidase enzyme will catalyze the hydrolysis of α-D-galactosidic bonds contained in the hemicellulose galacto-oligosaccharides [24]. EM4 (Effective Microorganisms) consists of three main components, *Rhodopseumonas palustris, Lactobacillus casei,* and *Saccharomyces cerevisiae*. *R. palustris* is able to degrade aromatic compounds such as coumaroyl amide, feruloyl amide, coumaric acid and benzoic acid contained in lignin through the benzoyl-CoA pathway. The decomposition of lignocellulosic materials will produce aromatic compounds which will inhibit the hydrolysis and fermentation processes. *R. palustris* can degrade aromatic compounds anaerobically without destroying or consuming glucose and xylose which are required during the fermentation process [25].

Table 10. The result of reducing sugar produced from the use of four different catalysts [21]

Treatment	Concentration of reducing sugar (g/100 ml)
Cellulose	8.0667
EM4	8.7900
Multienzyme	11.0267
Control	4.700

From table 10, it can be seen that multienzyme produced higher reducing sugar levels (11.0267 g/100 ml), compared to cellulose (8.0667 g/100 ml) and EM4 (8.7900 g/100 ml). The lowest production of reducing sugar was found in the control treatment (4.700 g/100 ml). Reducing sugar affected the yield of ethanol, the higher concentration of reducing sugar, the higher the concentration of ethanol. After the hydrolysis process using various enzymes, the ethanol production process was carried out by adding *Saccharomyces cerevisiae* with 3 various concentrations. The result of the addition of *S. cerevisiae* showed different ethanol yields, which can be seen in Table 11.

Table 11. Ethanol content produced based on the duration of fermentation using different catalysts with different concentrations of *Saccharomyces cerevisiae* [21]

Fermentation time	EM4			Multienzyme			Cellulose			Control		
	1%	2%	3%	1%	2%	3%	1%	2%	3%	1%	2%	3%
2 days	2.50	3.15	3.27	3.14	3.76	3.66	2.38	2.63	3.17	1.72	2.00	1.70
4 days	2.46	2.89	2.63	2.63	3.24	3.42	2.43	2.45	2.61	2.38	2.57	2.34
6 days	2.48	2.22	2.73	2.73	3.21	3.45	2.09	2.22	2.50	1.81	2.23	1.90

The variation in yeast concentration, the use of 2% or 3% yeast can increase the production of ethanol content, compared to the use of 1% yeast. In the hydrolysis process, the highest ethanol content was found on the 2^{nd} day, after which the ethanol concentration decreased. The high concentration of ethanol on the 2^{nd} day occured due to the yeast cells entered the exponential phase. In this phase, the yeast cells will produce ethanol and grow rapidly [21]. The highest percentage of ethanol content from the fermentation process was 3.76% with the addition of 2% yeast (*S. cerevisiae*) on the 2^{nd} day using multienzyme. EM4 produced the highest ethanol at the addition of 3% yeast, which was 3.27% ethanol. The highest ethanol production with the addition of cellulose using 3% yeast was 3.17%. The control showed 2.57% ethanol yield using 2% yeast.

V. Conclusion

From several articles that have been discussed, it can be concluded that bioethanol can be produced from cassava peels which in general have not been utilized optimally. Bioethanol derived from cassava peels can be used as an alternative to renewable natural resources. The materials required in the production of bioethanol from cassava peels are cheap and easily obtainable. This has the potential to be a source of wealth for tropical countries if utilized optimally.

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