

Temperature and pH effects on bioethanol production from wild cassava (*Manihot glaziovii* Muell. Arg) using simultaneous co-fermentation

Ida Ayu Pridari Tantri¹, Ida Bagus Wayan Gunam^{1,2}, Anak Agung Made Dewi Anggreni^{1,2},
I Gede Arya Sujana²

¹Department of Agroindustrial Technology, Faculty of Agricultural Technology, Udayana University, Badung, Indonesia

²Laboratory of Bioindustry, Faculty of Agricultural Technology, Udayana University, Badung, Indonesia

Article Info

Article history:

Received Nov 26, 2025

Revised Dec 10, 2025

Accepted Jan 1, 2026

Keywords:

Bioethanol

pH

Simultaneous co-fermentation

saccharification

Temperature

Wild cassava

ABSTRACT

Bioethanol is a clean alternative energy source, with wild cassava (*Manihot glaziovii* Muell. Arg) as a potential feedstock. Fermentation converts glucose from hydrolysis into ethanol. This study examines the effects of pH and fermentation temperature on bioethanol characteristics using a simultaneous saccharification and co-fermentation (SSCF) technique. A factorial randomized block design (RBD) was used with two factors: pH (4.5, 5.0, and 5.5) and fermentation temperature (30, 32.5, and 35 °C). Data were analyzed using variance and Duncan's test. Results showed that pH and temperature significantly affected pH value, total soluble solids, reducing sugar, and ethanol content. The optimal conditions for bioethanol production were pH 4.5 and temperature 32.5 °C, yielding a pH of 3.55±0.07, total soluble solids of 9.3±0.57 °Brix, reducing sugar of 3.038±0.10 mg/mL, and ethanol content of 3.48±0.37 (%w/v). Based on the results of this study, wild cassava can be utilized as bioethanol by considering the effect of fermentation conditions.

This is an open access article under the [CC BY-SA](https://creativecommons.org/licenses/by-sa/4.0/) license.



Corresponding Author:

Ida Bagus Wayan Gunam

Department of Agroindustrial Technology, Faculty of Agricultural Technology, Udayana University

Bukit Jimbaran, Badung Bali-80361, Indonesia

Email: ibwgunam@unud.ac.id

1. INTRODUCTION

Energy has emerged as an important issue recently in the world, affecting the economic development along with the growth of population, fuel emission problems, and not to mention depletion of oil reserves. The relentlessly increasing consumption of fossil energy has triggered the crisis of fuel [1]. The scarcity and impact of environmental pollution due to the use of fossil fuels has encouraged many innovations to find renewable and environmentally friendly alternative fuels. The solution to energy problems and the greenhouse effect caused by the use of fossil fuels is the development of alternative fuels. One of these alternative fuels is bioethanol [2]. Bioethanol is an alternative that will be needed in the future, and its production will increase significantly because of the large number of raw materials available to produce bioethanol [3].

Bioethanol production as an alternative energy source is nothing new. Bioethanol production currently still uses food raw materials necessary for human life, such as corn, beans, and tubers such as cassava [4]. Bioethanol production from this material can solve energy problems, but if implemented on a large scale, it will create new problems, such as food shortages for human life. One of the inedible natural materials that has the potential to be used as a raw material for making bioethanol is wild cassava (*Manihot glaziovii* Muell. Arg).

Wild cassava is a non-food ingredient because it contains cyanide acid (hydrogen cyanide (HCN)), which is toxic if consumed directly, so it is not generally sold [5]. Wild cassava is mostly composed of starch, which is around 78% of the dry weight. Other components of wild cassava include water, protein, fat, fiber, and ash. Wild cassava fiber consists of cellulose, hemicellulose, lignin, and protein, so raw cassava can be used as a raw material for making bioethanol [6]. The yield of wild cassava in the bioethanol production process is very dependent on the ability of the process to hydrolyze wild cassava components, especially starch into glucose. The high or low content of hydrolyzed glucose will influence the bioethanol formation process in the fermentation process [6].

Bioethanol in this study was produced using several main process stages, namely gelatinization, simultaneous saccharification and co-fermentation (SSCF), and distillation. The enzymatic hydrolysis process can be carried out simultaneously with co-fermentation of glucose, cellulose, and xylose, which is called SSCF [7]. The advantage of SSCF is that production costs can be reduced, the enzymatic hydrolysis process can be eliminated, and the productivity of ethanol production will be higher than the separate hydrolysis and fermentation processes [8]. Co-fermentation requires an engineering process of microorganisms to create optimal conditions during fermentation, thereby increasing ethanol yield. The SSCF process technology is a single fermenter fermentation process that can shorten production time and reduce production costs without the addition of pure enzymes for hydrolysis. The SSCF process can be used to produce bioethanol from wild cassava starch using microorganisms that produce extracellular enzymes. Enzyme activity is influenced by several factors, namely temperature, pH, substrate concentration, enzyme concentration, and the presence of inhibitors [6]. According to Salihu *et al.* [9], temperature and pH are the most important factors to be known in the fermentation process because each enzyme functions optimally at a certain temperature and pH. Above the optimal temperature, the reaction rate decreases sharply because enzymes are proteins that denature at high temperatures.

Several studies on bioethanol from wild cassava using the simultaneous saccharification and fermentation (SSF) and SSCF methods reported that the SSF process using cassava substrate with the help of the microorganisms *Aspergillus niger* and *Saccharomyces cerevisiae* can produce up to 8% bioethanol using a raw cassava substrate with a pH of 5.0 and fermentation temperature 35 °C produced ethanol levels of 4.30 g/100 mL [10]. Other researchers have reported that bioethanol can be produced from raw cassava by co-cultivating 10% *Aspergillus niger*, *Rhizopus oligosporus*, *Trichoderma reesei*, and *Saccharomyces cerevisiae* with a pH of 5.0 and a fermentation temperature of 30 °C to produce an ethanol content of 12.9 g/L [11]. The SSCF process uses rice straw as a substrate with the help of *Saccharomyces cerevisiae* microorganisms and xylanase enzymes with a pH of 4.8 and a temperature of 34 °C, which can produce an ethanol content of 0.35 w/w [12]. Recently, Gunam *et al.* [6] reported that the highest ethanol yield (28.277±0.228 g/L) was obtained from the wild cassava flour (WCF) substrate fermented using a co-culture of R5I4 isolate, *Aspergillus niger*, and R5I3 isolate after 8 days of incubation.

In this research, the bioethanol production process was carried out using the SSCF technique using local microbials *Candida orthopsilosis* R5I3 and *Klebsiella variicola* R5I4 isolated in previous research [6] and *Aspergillus niger* Food and Nutrition Culture Collection (FNCC) 6018. This study examined the influence of pH and fermentation temperature in the bioethanol production process from wild cassava using the SSCF technique. The purpose of this research is to: i) determine the effect of pH and fermentation temperature on the characteristics of bioethanol from WCF using the SSCF technique; and ii) determine the appropriate pH and fermentation temperature to produce bioethanol from WCF using a SSCF technique with the best characteristics.

2. RESEARCH METHOD

2.1. Research design

The experimental design in this study used a factorial randomized block design (RBD) with two factors. The first factor was the initial pH (P), which consists of three levels, namely 4.5, 5.0, and 5.5. The second factor was fermentation temperature (T), which consists of three levels, namely 30, 32.5, and 35 °C.

Based on the combination of the two factors above, nine treatments were obtained, and each treatment was grouped into two groups based on processing time, so that eighteen treatment units were obtained. The main ingredient used was wild cassava, which was obtained from Karangasem District, Karangasem Regency, Bali, Indonesia. The wild cassava obtained was processed into WCF through a process of cutting, soaking, drying, grinding, and sieving. The following additional materials were utilized in this study: *Aspergillus niger* FNCC 6018, along with local microbes *Candida orthopsilosis* R5I3 and *Klebsiella variicola* R5I4, which were obtained from the Bioindustry Laboratory at the Department of Agroindustrial Technology, Faculty of Agricultural Technology, Udayana University.

2.2. Research procedure

2.2.1. Preparation materials

Wild cassava tubers were peeled, cut into smaller pieces, washed thoroughly, soaked and continuously stirred for approximately 72 h with water was replaced periodically every 24 h. The tubers were then sun-dried, then dried using an oven at 85 °C for 5-7 h. The dried wild cassava pieces were then ground using a blender and sieved with a 60-mesh sieve.

2.2.2. Microorganisms and culture medium

Candida orthopsilosis R5I3 and *Klebsiella variicola* R5I4 were rejuvenated using yeast extract peptone glucose (YPG) medium with a composition of 0.45% yeast extract, 0.75% peptone, and 5% glucose in distilled water [3], [6], [13]. Three milliliters of each isolate culture were inoculated into 50 mL that had been sterilized and incubated at 35 °C for 24 h. Propagation isolates are re-inoculated into 250 mL of liquid YPG medium for 24 h at room temperature and 125 rpm agitation for cell growth. After the growing culture is obtained, it is centrifuged, and the pellet is collected [3], [13].

Aspergillus niger FNCC 6018 was rejuvenated and propagated using potato dextrose broth (PDB) media. At the rejuvenation stage, the culture was incubated for 48-72 h at 35 °C in an incubator. Then transferred to new PDB media for propagation and incubated for 96 h, at 125 rpm, at room temperature ± 30 °C, it will produce active cells of *Aspergillus niger* FNCC 6018 [14].

2.2.3. Single-stage of SSCF in one single bioreactor

SSCF carried out refers to the procedure used by Bermejo *et al.* [15] and Moshi *et al.* [16]. The initial stage begins with weighing 100 g of WCF and adding 700 mL of distilled water, or using a ratio of 1:7 for one fermentation medium. Gelatinization at 80 °C to form a thick slurry, adjust the substrate pH to pH 4.5, 5.0, and 5.5 using citrate buffer, and transfer 200 mL of substrate into a 250 mL fermentor. Sterilization using an autoclave at 121 °C for 15 min with a pressure of 1 atm, after which it was cooled to room temperature. *Aspergillus niger* FNCC 6018 and *Klebsiella variicola* R5I4 were added simultaneously before the fermentation process on day 0 and after four days of the fermentation process *Candida orthopsilosis* R5I3 was added to the substrate with a treatment concentration of 2% in each experimental unit. The fermentation was performed in a waterbatch shaker at 30, 32.5, and 35 °C with a stirring speed of 100 rpm for 8 h. The fermentation was then filtered and analyzed.

The distillation process in this research refers to the research procedure of Gunam *et al.* [6] with modifications. After the fermentation process, a filtration process was performed using a distillator to separate the fermentation filtrate and the solids. The filtrate was measured with a graduated cylinder, and then a multistage distillation was performed at a temperature of 100 °C for the first bottle and 80 °C for the second bottle. After distillation, the ethanol content was analyzed.

2.3. Analytical methods

The variables observed in this research include, in the initial stage before fermentation, pH analysis was carried out using a pH meter to measure the initial pH before fermentation [17], and total soluble solids analysis was carried out using a hand refractometer to measure the initial total soluble solids [18]. After the fermentation process, pH analysis, total soluble solids analysis, and reducing sugar analysis are performed using a ultraviolet-visible (UV-Vis) spectrophotometer to measure the results of reducing sugar produced during the fermentation process [6], [13]. Then, after the distillation process, ethanol content analysis was performed using an alcohol meter to determine the ethanol produced [13]. In general, the flow diagram of the bioethanol production experiment is presented in Figure 1 (see Appendix).

2.4. Data analysis

Data were analyzed using the parametric analysis of variance (ANOVA) test at a significance level of $p < 0.05$. When significant differences were observed among treatments, further analysis was conducted using Duncan's multiple range test (DMRT). All statistical processing was performed with the Minitab software program.

3. RESULTS AND DISCUSSION

3.1. pH

The results of variance analysis showed that pH, fermentation temperature, and their interactions had a very significant effect ($p < 0.01$) on the pH value produced during the SSCF process. The average pH value in the fermentation process can be seen in Table 1. The results in Table 1 show the average pH value for each pH treatment and fermentation temperature. The highest decrease in average pH for fermentation

results occurred in the pH 4.5 treatment with a fermentation temperature of 35 °C, resulting in a pH of 3.73 ± 0.11 . The lowest pH value occurred in the pH 5.5 treatment with a temperature of 30 °C yields 3.18 ± 0.04 , but was not significantly different from pH 5.0 at 30 °C, and pH 5.5 at 32.5 °C and 35 °C. From these results, the pH in each treatment increased during the fermentation process due to the by-products of the fermentation process in the form of organic acids. This is supported by the statement of Ma *et al.* [19] that the fermentation process produces by-products in the form of organic acids, oligosaccharides, amylase, lipase, cellulase, xylase, and protease. This statement is reinforced by the view of Ghori *et al.* [20], which states that the fermentation process not only produces ethanol but also organic acid compounds, thereby increasing the acidity of the substrate.

Table 1. Average pH value of fermentation results

pH	Temperature (°C)		
	30 °C	32.5 °C	35 °C
4.5	3.38 ± 0.11^{cd}	3.55 ± 0.07^b	3.73 ± 0.11^a
5.0	3.28 ± 0.04^{de}	3.38 ± 0.11^{cd}	3.45 ± 0.07^{bc}
5.5	3.18 ± 0.04^e	3.23 ± 0.04^{de}	3.30 ± 0.00^{de}

Note: means followed by different letters within the same row or column are significantly different ($p < 0.05$)

In general, pH is an important factor in the fermentation process because it influences the activity of microorganisms [21], and proper pH is the key to microbial growth and metabolism [22]. According to Gunam *et al.* [6], the optimal pH value for R513 and R514 activity is pH 5. Other research states that the optimal pH for the active enzyme-producing enzymes from *Aspergillus niger* is pH 5-5.5 [23]. Research by Turhan *et al.* [24] stated that pH-controlled treatments were able to produce higher ethanol concentrations than treatments without pH control. The initial pH value of this study was set at pH variations of 4.5, 5.0, and 5.5 in order to create an optimal fermentation medium for the microorganisms.

3.2. Total soluble solids

The results of the ANOVA showed that the pH and fermentation temperature treatments had a real effect ($p > 0.05$), while the pH and interaction had no significant effect ($p > 0.05$) on the total value of soluble solids produced during the SSCF process. The average value of total soluble solids in the fermentation process can be seen in Table 2. The results in Table 2 show the average total soluble solids occurring at each pH and fermentation temperature. The average value of total soluble solids is 9.3–7.9 °Brix. The treatment of pH 5.5 was not significantly different from pH 4.5 and 5.0. The highest temperature treatment was at 32.5 °C, but not significantly different from 35 °C, and lowest was at 35 °C. In the fermentation process, sugar is metabolized by R513 into alcohol and CO₂, causing low total soluble solids. As stated by Sujana *et al.* [13], the low total soluble solids are thought to be because during the fermentation process, sugar, which is the dominant solid component in the medium, is metabolized by yeast into alcohol and CO₂, then used as a carbon source by lactic acid bacteria (LAB), so that the total solids content dissolved to low. This is reinforced by the statement of Gunam *et al.* [6], where the decrease in total soluble solids during storage is due to the conversion of the sugar contained into alcohol, aldehydes, and amino acids. This opinion is supported by Gunam *et al.* [25] stated that the lower the total solids content, the higher the ethanol content.

The decrease in total soluble solids also occurred due to the breakdown of glucose by microbes into alcohol. The added microorganisms have the ability to produce α -amylase and glucoamylase, which are used to convert the amylose and amylopectin contained in starch into sugars (galactose, fructose, and glucose) during aerobic or anaerobic fermentation, converted into ethanol, CO₂, and other by-products [26]. The fungal microorganism *Aspergillus niger* is capable of producing glucoamylase, cellulase, xylanase, amylase, protease, and lipase [27], [28]. This ability is required in the fermentation process to convert starch to ethanol.

Table 2. Average value of total soluble solids from fermentation (°Brix)

pH	Temperature (°C)			Average
	30 °C	32.5 °C	35 °C	
4.5	7.90 ± 0.42	9.30 ± 0.57	8.05 ± 0.35	8.42 ± 0.11^a
5.0	7.95 ± 0.07	9.30 ± 0.42	8.60 ± 0.28	6.71 ± 0.18^a
5.5	8.60 ± 0.28	8.40 ± 0.85	8.65 ± 0.21	6.63 ± 0.35^a
Average	8.15 ± 0.18^a	9.0 ± 0.22^a	8.43 ± 0.07^{ab}	

Note: means followed by different letters within the same row or column are significantly different ($p < 0.05$)

3.3. Reducing sugar

The results of variance analysis showed that pH, fermentation temperature, and interactions had a very significant effect ($p < 0.01$) on the value of reducing sugar produced during the SSCF process. The average value of reducing sugar in the fermentation process can be seen in Table 3. The results in Table 3 show the average value of reducing sugar for each pH and fermentation temperature treatment. The highest average value of reducing sugar resulting from fermentation occurred in the pH 5.5 treatment with a fermentation temperature of 32.5 °C, producing 3.66±0.12 mg/mL. The lowest average value of reducing sugar resulting from fermentation occurred in the pH 4.5 treatment with a fermentation temperature of 30 °C, producing 0.94±0.03 mg/mL. With increasing pH and fermentation temperature, the value of reducing sugar produced by the fermentation test becomes higher, which shows that the process of breaking down starch into simple sugars continues. Therefore, there is more reducing sugar used by yeast, the higher the concentration of ethanol it can produce, and conversely, the less reducing sugar used by yeast, the lower the concentration of ethanol it can produce [29].

Table 3. Average value of reducing sugar from fermentation

pH	Temperature (°C)		
	30 °C	32.5 °C	35 °C
4.5	0.94±0.03 ^g	3.04±0.10 ^b	1.60±0.18 ^e
5.0	1.22±0.20 ^f	2.33±0.10 ^d	2.42±0.12 ^{cd}
5.5	1.69±0.12 ^e	3.66±0.12 ^a	2.60±0.18 ^c

Note: means followed by different letters within the same row or column are significantly different ($p < 0.05$)

The microorganisms *Aspergillus niger*, *Candida orthopsilosis*, and *Klebsiella variicola* play an important role in producing α -amylase and glucoamylase to degrade starch into simple sugars. Starch is divided into amylose and amylopectin. Amylose consists of α -1,4 glycosidic bonds, and amylopectin consists of α -1,6 glycosidic bonds. The α -1,4 glycosidic bonds in starch amylose react with α -amylase during liquefaction to produce reducing sugars [30]. Glucoamylase in amylopectin starch hydrolyzes α -1,4 glycosidic bonds and α -1,6 glycosidic bonds into monosaccharide units, a process called saccharification [30]. Fermented glucose is utilized by microorganisms as a source of nutrition and produces ethanol by-products and organic acids [6], [13].

3.4. Ethanol content

The results of the ANOVA showed that the pH and fermentation temperature had a very significant effect ($p < 0.01$), while the interaction had a significant effect ($p < 0.05$) on the value of the ethanol content produced during the SSCF process. The average value of ethanol content in the fermentation process can be seen in Table 4. The results in Table 4 show the average value of ethanol content occurring at each pH and fermentation temperature. The highest average ethanol value was at pH 4.5 with a fermentation temperature of 32.5 °C, producing 3.48±0.37% w/v, not significantly different from pH 4.5 at 30 °C and pH 5.0 at 32.5 °C. The lowest average ethanol value was at pH 5.5 with a fermentation temperature of 32.5 °C, producing 1.35±0.24% w/v, but was not significantly different from pH 5.0 at 30 °C and 35 °C. The ethanol content value tends to decrease as the pH value increases. This shows that at pH 5.5, the growth and activity of R5I3 are not optimal. R5I3 enters the stationary phase or becomes less efficient at pH 5.5. This phase is caused by the level of glucose used as a carbon source for yeast cells tending to decrease, preventing the yeast from breaking down into ethanol and causing some of the yeast to die [31]. If the pH is increased, the growth rate will decrease, and eventually, growth will stop altogether. Growth arrest can be caused by a reduction in several essential nutrients in the medium, or by the accumulation of autotoxins in the medium, or a combination of both [13]. Meanwhile, the ethanol content value tends to increase with increasing fermentation temperature.

Table 4. Average value of ethanol content (% w/v)

pH	Temperature (°C)		
	30 °C	32.5 °C	35 °C
4.5	2.78±0.42 ^{abc}	3.48±0.37 ^a	2.37±0.50 ^{cd}
5.0	2.00±0.24 ^{de}	3.23±0.65 ^{ab}	2.11±0.26 ^{cde}
5.5	2.58±0.49 ^{bcd}	1.35±0.24 ^e	2.34±0.01 ^{cd}

Note: means followed by different letters within the same row or column are significantly different ($p < 0.05$)

The optimum temperature for growth and activity of *Aspergillus niger* is 30 °C. The optimum temperature for *Candida orthopsilosis* R5I3 and *Klebsiella variicola* R5I4 growth and activity is 30-35 °C. However, in this study, it was slightly different; at a temperature of 32.5 °C, the microbes could still work, and this was the best result for producing the highest ethanol. Temperature plays an important role because it can directly affect the microbial activity and indirectly affect the amount of ethanol produced [32]. Apart from that, the decrease in bioethanol concentration was also influenced by the decrease in sugar concentration and the hydrolysis process, which was slower than the fermentation rate. When the fermentation rate is fast while there is a shortage of sugar substrates, some yeasts tend to consume ethanol, causing a further reaction of oxidized ethanol to become acetic acid Hata *et al.* [33].

4. CONCLUSION

Recent observations indicate that the treatment of pH and fermentation temperature had a significant effect on the pH value, reduced sugar value, and ethanol content value, but had no significant effect on the total value of bioethanol soluble solids from WCF using the SSCF technique. The pH treatment of 4.5 with a fermentation temperature of 32.5 °C is the right pH and fermentation temperature to produce bioethanol from WCF with the following characteristics of fermentation results: pH value 3.55±0.07; total soluble solids value 9.3±0.57 °Brix; reduced sugar value 3.038±0.10 mg/mL; and ethanol content value 3.48±0.37% w/v. A re-examination of the results of this study is necessary, given the need to employ diverse fermentation conditions to achieve optimal outcomes. Consequently, wild cassava emerges as a promising candidate for consideration as an alternative raw material in bioethanol production.

ACKNOWLEDGEMENTS

The authors would like to thank Udayana University through the Institute for Research and Community Service for funding this research, and the Faculty of Agricultural Technology for its laboratory facilities, especially the Bioindustry Laboratory.

FUNDING INFORMATION

This research was funded by Udayana University through the “Penelitian Invensi Udayana (PIU)” scheme with grant number B/78.567/UN14.4.A/PT.01.03/2022.

AUTHOR CONTRIBUTIONS STATEMENT

This journal uses the Contributor Roles Taxonomy (CRediT) to recognize individual author contributions, reduce authorship disputes, and facilitate collaboration.

Name of Author	C	M	So	Va	Fo	I	R	D	O	E	Vi	Su	P	Fu
Ida Ayu Pridari Tantri		✓	✓	✓	✓	✓		✓	✓		✓			
Ida Bagus Wayan Gunam	✓	✓		✓			✓		✓	✓	✓	✓	✓	✓
Anak Agung Made Dewi Anggreni	✓									✓		✓	✓	
I Gede Arya Sujana			✓		✓	✓	✓	✓		✓	✓			

C : **C**onceptualization

M : **M**ethodology

So : **S**oftware

Va : **V**alidation

Fo : **F**ormal analysis

I : **I**nvestigation

R : **R**esources

D : **D**ata Curation

O : Writing - **O**riginal Draft

E : Writing - Review & **E**ditting

Vi : **V**isualization

Su : **S**upervision

P : **P**roject administration

Fu : **F**unding acquisition

CONFLICT OF INTEREST STATEMENT

The authors declare no potential conflicts of interest, financial or non-financial, including any funding sources, personal relationships, professional affiliations, or ideological interests that could influence the research findings. This work was conducted independently to maintain objectivity and integrity in reporting results related to wild cassava (*Manihot glaziovii* Mull. Arg.) processing and analysis.

DATA AVAILABILITY

All data generated or analyzed during this study are included in this published article.

REFERENCES

- [1] N. Handra, A. Kasim, G. Gunawarman, and S. Santosa, "Effect of heating temperature on quality of bio-briquette empty fruit bunch fiber," *International Journal of Advances in Applied Sciences*, vol. 9, no. 3, pp. 192–200, Sep. 2020, doi: 10.11591/ijaas.v9.i3.pp192-200.
- [2] M. Krajang, K. Malairuang, J. Sukna, K. Rattanapradit, and S. Chamsart, "Single-step ethanol production from raw cassava starch using a combination of raw starch hydrolysis and fermentation, scale-up from 5-L laboratory and 200-L pilot plant to 3000-L industrial fermenters," *Biotechnology for Biofuels*, vol. 14, no. 1, Dec. 2021, doi: 10.1186/s13068-021-01903-3.
- [3] I. B. W. Gunam, I. G. A. Sujana, I. M. M. Wijaya, Y. Setiyo, I. W. W. P. Putra, and L. Suriati, "Isolation and selection of amylase-producing microbes isolated from *ragi tape* and cassava *tape* available on the markets," *IOP Conference Series: Earth and Environmental Science*, vol. 913, no. 1, Nov. 2021, doi: 10.1088/1755-1315/913/1/012041.
- [4] N. H. Nabil, P. S. B. Handoko, F. W. Destantri, A. B. Syahputra, and Z. A. S. Bahlawan, "Bioethanol production from rice straw through utilization of Agrobiomass Waste in Central Java towards clean energy: a review," *Journal of Clean Technology*, vol. 1, no. 1, pp. 1–8, Feb. 2024, doi: 10.15294/joct.v1i1.1656.
- [5] P. Dhas, P. Chitra, S. Jayakumar, and A. Mary, "Study of the effects of hydrogen cyanide exposure in cassava workers," *Indian Journal of Occupational and Environmental Medicine*, vol. 15, no. 3, pp. 133–136, 2011, doi: 10.4103/0019-5278.93204.
- [6] I. B. W. Gunam, I. G. A. Sujana, I. M. M. Wijaya, I. W. Arnata, Y. Setiyo, and I. W. W. P. Putra, "Experimentation of various co-culture fermentation strategies for better efficiency bioethanol production using wild cassava flour (*Manihot glaziovii* Muell. Arg) as substrate," *South African Journal of Chemical Engineering*, vol. 55, pp. 271–284, Jan. 2026, doi: 10.1016/j.sajce.2025.11.013.
- [7] X. Yuan *et al.*, "Modified simultaneous saccharification and co-fermentation of DLC pretreated corn stover for high-titer cellulosic ethanol production without water washing or detoxifying pretreated biomass," *Energy*, vol. 247, May 2022, doi: 10.1016/j.energy.2022.123488.
- [8] Ritika *et al.*, "Simultaneous fermentation of glucose and xylose by using co-culture of *S. cerevisiae* and a potential robust pentose fermenting fungi (*Fusarium incarnatum*)," *Biomass Conversion and Biorefinery*, vol. 13, no. 9, pp. 8089–8100, Jun. 2023, doi: 10.1007/s13399-021-01801-6.
- [9] U. Y. Salihu, U. G. Usman, A. Y. Abubakar, and G. Mansir, "Effect of pH and temperature on bioethanol production: evidences from the fermentation of sugarcane molasses using *Saccharomyces cerevisiae*," *Dutse Journal of Pure and Applied Sciences*, vol. 8, no. 4b, pp. 9–16, Jan. 2023, doi: 10.4314/dujopas.v8i4b.2.
- [10] S. Ado, G. Olukotun, J. Ameh, and A. Yabaya, "Bioconversion of cassava starch to ethanol in a simultaneous saccharification and fermentation process by co-cultures of *Aspergillus niger* and *Saccharomyces cerevisiae*," *Science World Journal*, vol. 4, no. 1, Feb. 2010, doi: 10.4314/swj.v4i1.51832.
- [11] N. Kagwanwong, C. Sakdaronnarong, P. Prayoonyon, and W. Jonglertjunya, "Bioconversion of cassava root to ethanol by co-cultivation of amylolytic enzyme production mould and *Saccharomyces cerevisiae*," *Kasem Bundit Engineering Journal*, vol. 8, pp. 112–121, 2018.
- [12] K. Olofsson, B. Palmqvist, and G. Lidén, "Improving simultaneous saccharification and co-fermentation of pretreated wheat straw using both enzyme and substrate feeding," *Biotechnology for Biofuels*, vol. 3, no. 1, Dec. 2010, doi: 10.1186/1754-6834-3-17.
- [13] I. G. A. Sujana, I. B. W. Gunam, I. M. M. Wijaya, and I. W. W. P. Putra, "Producing ethanol from coconut sap using yeast isolates R513, IS258 and *Saccharomyces cerevisiae* ATCC 9763 and the optimal fermentation time required," *IOP Conference Series: Earth and Environmental Science*, vol. 1271, no. 1, Dec. 2023, doi: 10.1088/1755-1315/1271/1/012064.
- [14] G. B. Santos, Á. de S. F. Filho, J. R. da S. Rodrigues, and R. R. de Souza, "Cellulase production by *Aspergillus niger* using urban lignocellulosic waste as substrate: evaluation of different cultivation strategies," *Journal of Environmental Management*, vol. 305, Mar. 2022, doi: 10.1016/j.jenvman.2022.114431.
- [15] F. J. R.-Bermejo, M. R.-Bailen, J. G.-Cebrian, and M. J. H.-Ranchal, "Sepsis-induced cardiomyopathy," *Current Cardiology Reviews*, vol. 7, no. 3, pp. 163–183, Aug. 2011, doi: 10.2174/157340311798220494.
- [16] A. P. Moshi *et al.*, "Combined production of bioethanol and biogas from peels of wild cassava *Manihot glaziovii*," *Chemical Engineering Journal*, vol. 279, pp. 297–306, Nov. 2015, doi: 10.1016/j.cej.2015.05.006.
- [17] B. Traynor, H. Uvegi, E. Olivetti, B. Lothenbach, and R. J. Myers, "Methodology for pH measurement in high alkali cementitious systems," *Cement and Concrete Research*, vol. 135, Sep. 2020, doi: 10.1016/j.cemconres.2020.106122.
- [18] I. Handayani, A. T. Septiana, P. Haryanti, B. Sustriawan, and S. B. Sulisty, "Evaluation of colour and physicochemical properties of annatto seed aquadest extract in the variation pH of solvent," in *The Third International Symposium on Food and Agrobiodiversity (ISFA 2021)*, 2023, doi: 10.1063/5.0108054.
- [19] X. Ma, M. Gao, N. Wang, S. Liu, Q. Wang, and X. Sun, "Lactic acid production from co-fermentation of food waste and spent mushroom substance with *Aspergillus niger* cellulase," *Bioresource Technology*, vol. 337, Oct. 2021, doi: 10.1016/j.biortech.2021.125365.
- [20] M. I. Ghoris, S. Ahmed, M. A. Malan, and A. Jamil, "Corn stover-enhanced cellulase production by *Aspergillus niger* NRRL 567," *African Journal of Biotechnology*, vol. 10, no. 31, Jun. 2011, doi: 10.5897/AJB10.2342.
- [21] R. Sharma, P. Garg, P. Kumar, S. K. Bhatia, and S. Kulshrestha, "Microbial fermentation and its role in quality improvement of fermented foods," *Fermentation*, vol. 6, no. 4, Nov. 2020, doi: 10.3390/fermentation6040106.
- [22] S. R. Chaganti, D.-H. Kim, J. A. Lalman, and W. A. Shewa, "Statistical optimization of factors affecting biohydrogen production from xylose fermentation using inhibited mixed anaerobic cultures," *International Journal of Hydrogen Energy*, vol. 37, no. 16, pp. 11710–11718, Aug. 2012, doi: 10.1016/j.ijhydene.2012.05.036.
- [23] I. Ahmed, M. A. Zia, M. A. Hussain, Z. Akram, M. T. Naveed, and A. Nowrouzi, "Bioprocessing of citrus waste peel for induced pectinase production by *Aspergillus niger*; its purification and characterization," *Journal of Radiation Research and Applied Sciences*, vol. 9, no. 2, pp. 148–154, Apr. 2016, doi: 10.1016/j.jrras.2015.11.003.
- [24] I. Turhan, K. L. Bialka, A. Demirci, and M. Karhan, "Ethanol production from carob extract by using *Saccharomyces cerevisiae*," *Bioresource Technology*, vol. 101, no. 14, pp. 5290–5296, Jul. 2010, doi: 10.1016/j.biortech.2010.01.146.
- [25] I. B. W. Gunam, T. E. B. Kaban, and N. P. Suwariani, "Effect of yeast concentration and fermentation time on the characteristics of tuak from coconut sap," *Canrea Journal: Food Technology, Nutritions, and Culinary Journal*, vol. 5, no. 2, pp. 139–150, Dec. 2022, doi: 10.20956/canrea.v5i2.599.

- [26] T. J. Tse, D. J. Wiens, and M. J. T. Reaney, "Production of bioethanol—a review of factors affecting ethanol yield," *Fermentation*, vol. 7, no. 4, Nov. 2021, doi: 10.3390/fermentation7040268.
- [27] L. Karaffa and C. P. Kubicek, "Production of organic acids by fungi," in *Encyclopedia of Mycology*, Elsevier, 2021, pp. 406–419, doi: 10.1016/B978-0-12-809633-8.21066-2.
- [28] S. E., D.-C. N., F. J., and P. van Dijck, "On the safety of *Aspergillus niger*-a review," *Applied Microbiology and Biotechnology*, vol. 59, no. 4–5, pp. 426–435, Jan. 2002, doi: 10.1007/s00253-002-1032-6.
- [29] C. N. Adewumi, E. I. Ekpo, O. Achugasim, R. E. Ogali, and O. Akaranta, "Substrate concentration: a more serious consideration than the amount of 5-hydroxymethylfurfural in acid-catalyzed hydrolysis during bioethanol production from starch biomass," *Heliyon*, vol. 8, no. 12, Dec. 2022, doi: 10.1016/j.heliyon.2022.e12047.
- [30] Y. Wang *et al.*, "Substrate selectivity of a novel Amylo- α -1,6-glucosidase from *Thermococcus gammatolerans* STB12," *Foods*, vol. 11, no. 10, May 2022, doi: 10.3390/foods11101442.
- [31] L. Galdieri, S. Mehrotra, S. Yu, and A. Vancura, "Transcriptional regulation in yeast during diauxic shift and stationary phase," *OMICS: A Journal of Integrative Biology*, vol. 14, no. 6, pp. 629–638, Dec. 2010, doi: 10.1089/omi.2010.0069.
- [32] X. Yan, P. Deng, T. Ding, Z. Zhang, X. Li, and Z. Wu, "Effect of temperature on anaerobic fermentation of poplar ethanol wastewater: performance and microbial communities," *ACS Omega*, vol. 8, no. 6, pp. 5486–5496, Feb. 2023, doi: 10.1021/acsomega.2c06721.
- [33] N. N. Y. Hata, M. Surek, D. Sartori, R. V. Serrato, and W. A. Spinosa, "Role of acetic acid bacteria in food and beverages," *Food Technology and Biotechnology*, vol. 61, no. 1, pp. 85–103, Nov. 2023, doi: 10.17113/ftb.61.01.23.7811.

APPENDIX

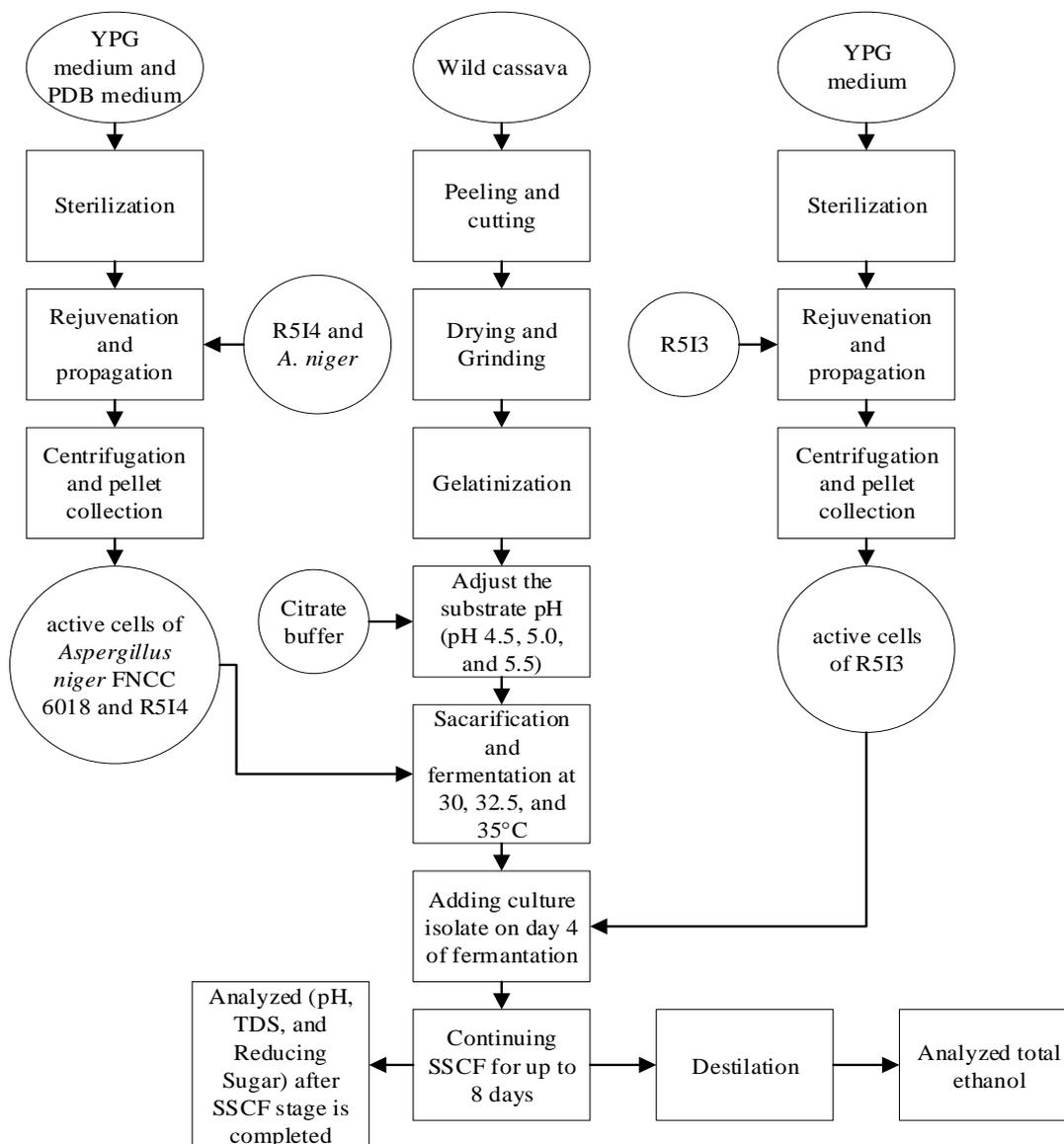


Figure 1. Flowchart of the various stages involved in the research process

BIOGRAPHIES OF AUTHORS

Ida Ayu Pridari Tantri    is a student of the Department of Agroindustrial Technology, Faculty of Agricultural Technology, Udayana University. Her research interests are bioenergy, environmental microbiology, and fermentation. She can be contacted at email: tantripridari@gmail.com.



Ida Bagus Wayan Gunam    is a senior lecturer in the Department of Agroindustrial Technology, Faculty of Agricultural Technology, Udayana University. He received his Ph.D. in 2006 from Hokkaido University, Japan. His research interests are bioenergy, environmental microbiology, and fermentation. He can be contacted at email: ibwgunam@unud.ac.id.



Anak Agung Made Dewi Anggreni    is a lecturer in the Department of Agroindustrial Technology, Faculty of Agricultural Technology, Udayana University. She received her doctorate in 2021 from Udayana University. Her research interest is microbiology, bioactive compounds, bioactivity, packaging, and storage technology. She can be contacted at email: dewiangreni@unud.ac.id.



I Gede Arya Sujana Sujana    is a master's graduate in Agricultural Biotechnology from Udayana University and currently works as a laboratory assistant at the Bioindustry Laboratory, Faculty of Agricultural Technology, Udayana University. His research interests include bioenergy and fermentation. He can be contacted at email: igedearyasujana2@gmail.com.