JEE Journal of Ecological Engineering

Journal of Ecological Engineering 2023, 24(1), 34–40
https://doi.org/10.12911/22998993/156000
ISSN 2299–8993, License CC-BY 4.0

Received: 2022.10.13
Accepted: 2022.11.09
Published: 2022.12.01

Bioethanol Production from *Chlorella Pyrenoidosa* by Using Enzymatic Hydrolysis and Fermentation Method

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ABSTRACT

Starch can be found in microalgae, the raw material for the third generation of bioethanol production. One of them is *C. pyrenoidosa*. This study was conducted to analyze the effect of α-amylase enzyme concentration on the glucose contents produced and the effects of fermentation time on bioethanol contents produced. The hydrolysis process of this study was conducted using an α-amylase enzyme produced by *A. niger*. Several analyses in this research were carried out, including the analysis of enzyme activity using the Fuwa method, the analysis of glucose levels from enzymatic hydrolysis using the DNS method, and the analysis of bioethanol contents using the density method and GC-MS. The highest glucose content was 0.67 mg/mL, which was obtained from the addition of 40% (v/w) α-amylase enzyme, and the yield of bioethanol content from the sample treated 40% (v/w) α-amylase enzyme and fermented for 9 days was the optimum, which produced 28.07% of bioethanol content.

Keywords: *Chlorella pyrenoidosa*, hydrolysis, Enzyme α-amylase, glucose, fermentation, bioethanol.

INTRODUCTION

Development in Indonesia has impacted the energy demand over the last decade. Fossil fuels, as scarce energy sources, must be replaced with renewable energy sources. Bioethanol is one alternative fuel that potentially reduces the dependence on fossil fuels. This fuel can be made from biomass, such as sweet potatoes (Rizzolo et al., 2021; Vitus & Otaraku, 2020), sago (Hung et al., 2018; Rijal, 2020), Maize (Tolulope Eunice, 2021), sorghum (Pandebesie et al., 2019) and sweet sorghum (Gallagher et al., 2018; Jiang et al., 2019; Klasson & Boone, 2021). The bioethanol produced by biomass is stated as first-generation, competing with biomass's function as food. Recently, many researchers produced bioethanol from non-food biomass that contained lignocellulose, such as waste crops and cereals (Melikoglu & Turkmen, 2019), rice straw (Gupta & Chundawat, 2020; Hassan et al., 2021), oil palm empty fruit bunches (Suhartini et al., 2022) and coconut husk (Bolivar-Telleria et al., 2018). The bioethanol produced by lignocellulose is stated as the second generation of bioethanol. The process of making bioethanol from lignocellulose carries a large energy cost because of the complex step process.

Bioethanol has been achieved in third-generation, which is made from algae and microalgae. Alga and microalgae have high potential as raw materials for bioethanol production due to uncomplicated cultivation and they can become a greater possible feedstock over the preceding generation (Goh & Lee, 2010). Furthermore, algae and microalgae do not need arable land and complex fertilization. Algae and microalgae only need sunlight, water, nutrient, and carbon dioxide for growth (Tan et al., 2020). Alga and microalgae-content carbohydrates can be hydrolyzed to become sugar; then, the sugar can be fermented to produce bioethanol. *C. pyrenoidosa* is one of the microalgae that potentially become the material for bioethanol

production. Besides being found in nature, *C. pyrenoidosa* is rarely used or has low aesthetic value. It also has a high carbohydrate content, around 37–55% (Moreira et al., 2019; Raheem et al., 2018; Zhao et al., 2019). The high content of carbohydrates made *C. pyrenoidosa* potentially become the alternative raw material for bioethanol through the hydrolysis and fermentation processes.

In this study, *C. pyrenoidosa* was hydrolyzed using α-amylase enzyme with various concentrations. The hydrolysis process using enzymes reduces carbohydrates into simple sugars with optimal results, unrequited costs, and an environment-friendly effect. The α-amylase enzyme can be found in *A. niger*. After the hydrolysis process, sugar was fermented using *S. cerevisiae* at various times. This yeast is the most effective microbe to convert sugar into bioethanol (Lee et al., 2017). Therefore, the study aimed to analyze the effects of α-amylase as a bio-catalyst in hydrolysis to produce sugar and the effects of fermentation time in producing bioethanol.

MATERIALS AND METHODS

Materials

C. pyrenoidosa is a type of microalgae widely available for several commercial applications in dietary and nutritional needs. *C. pyrenoidosa* has great potential as feedstock for the bioenergy industry in the future due to its fast growth rate and high carbohydrate and fat compounds (Dhull et al., 2014). Microalgae *C. pyrenoidosa* can produce carbohydrates, with fat as a reserve polymer. This species is ideal for bioethanol production, because glucose can be produced by extracting polysaccharides from algae. The carbohydrates derived from microalgae, particularly starch and cellulose, are easier to convert into monosaccharides than lignocellulosic materials. The high carbohydrate content of *C. pyrenoidosa* can be converted into a glucose compound by enzymatic or chemical hydrolysis and into alcohol by fermentation. *C. pyrenoidosa* has the highest carbohydrate content (26%) compared to other microalgae, such as *C. vulgaris* (51–58%), *Sp. platensis* (8–14%), *Sp. maxima* (13–16%), *E. gracilis* (14–18%) (Nur, 2014).

C. pyrenoidosa, as the raw material in this study, was hydrolyzed by using enzymatic hydrolysis. The enzyme used was a commercial α-amylase enzyme. α-amylase is an enzyme that can degrade starch, as a catalyst in hydrolysis by converting internal α-1-4 glycosidic bonds in polysaccharides to simpler sugars and limiting dextrin (Mojsov, 2016). The microorganisms that can produce heat-resistant amylase enzymes include *E. coli, B. subtilis, A. niger, A. oryzae, K. marxianus var*, and *S. cerevisiae* (Olempska-Beer et al., 2006). It was found that α-Amylase acts faster than β-Amylase because α-Amylase can do so in any substrate (Das et al., 2011). The use of α-amylase in this study was various. It was conducted to achieve the optimum α-amylase concentration for *C. pyrenoidosa* hydrolysis to obtain the maximum glucose product. The procees of bioethanol production wa showed at Figure 1.

Cultivation of *Chlorella pyronoidosa*

C. pyrenoidosa was cultivated in an open container filled with fresh water and equipped with an aerator as an oxygen supply medium with 3.5 L/min of airflow. The container was placed in a place exposed to direct sunlight for photosynthesis needs. The nutrients were fermented from a 70 g mixture of NPK, urea fertilizer, fishery EM4, rice bran, and sugar water in a ratio of 1:1:1:2:1 for 24 hours. Nutrients were provided as microalgae nutrients for their growth phase and given once during the 7-day growth process, with 20 L of *C. pyrenoidosa* seedlings. On the 6th day, fresh water was added with a ratio of 1:5. Then, *C. pyrenoidosa* was cultivated for a month before harvesting. After the cultivation process for a month, *C. pyrenoidosa* was harvested. The harvesting process was carried out using a 400-mesh plankton net. Then, *C. pyrenoidosa* dried in the sun for two days. Afterwards, the dry *C. pyrenoidosa* was ground using a mortar to make a powder. This study used 20 g of *C. pyrenoidosa* powder as a raw material.

Inoculation of *A. niger*

A. niger was inoculated with a loop sterilized needle and placed into the PDA medium by using a zigzag etching method. Then, *A. niger* was incubated at 30 °C for 7 days. Liquid media for A. niger was made using 100 mL of aquadest added with 12.5% sucrose ($C_{12}H_{22}O_{11}$), 0.25% $(NH_4)_2SO_4$, 0.2% KH_2PO_4 and added with HCl to adjust the pH-3. In this media, culture *A. niger* produced in PDA medium was added using a loop sterilized needle. It settled when the color became cloudy.

Making potato dextrose agar (PDA) medium

PDA medium was made from a mixture solution of 200 g of potatoes added with 600 mL of water. This mixed solution was boiled for 10 minutes and filtered using a 100-mesh sieve to separate the potato dregs and broth. Furthermore, the potato broth was added with 10 g of dextrose, and 10 g of agar, then stirred until homogeneous and heated at 60 °C for 20 min. This media was placed into a petri dish, filled half of the petri dish height, and closed tightly. Then, the media was sterilized by autoclave at 121 °C for 15 minutes. The sterile PDA medium was allowed to stand for 3 days at 30 °C until it became solid.

Production of the α-Amylase enzyme from *A. niger*

A sample of 20 g of *C. pyrenoidosa* microalgae and nutrients for *A.niger* such as 0.03 g urea ($CO(NH_2)_2$), 0.005 g $MgSO_4.7H_2O$, and 0.0023 g KH_2PO_4 was placed into a beaker glass and added 80 mL of aquadest. Then, 1 N HCl solution was added to adjust until the pH-5. The media was sterilized using the autoclave at 121 °C for 15 min, and added with *A. niger* about 10 mL of the liquid medium. Then, the media were incubated at 30 °C for 4 days. The incubation results were extracted using 100 mL of aquadest and homogenized with a rotary shaker at a speed of 150 rpm for 1 hour.

Furthermore, liquid and solid were separated by using a centrifuge. Afterwards, 1 mL of the enzyme solution was taken to analyze the α-amylase enzyme activity using the Fuwa method. The enzyme activity analysis began with the preparation of 0.1 N iodine solution using 1.269 g of iodine solids and 50 mL of K.I. solution added into the Erlenmeyer flask. Then, 50 mL of aquadest was added to the solution, and the K.I. solution could be added again until the iodine solid dissolves. The standard solution of the Fuwa method was prepared in the different Erlenmeyer flask with various starch 0.2, 0.4, 0.6, 0.8, and 1 g. each sample was dissolved in 100 mL aquadest for each concentration of 2, 4, 6, 8, and 1 mg/mL solutions. The standard solution of 1 mL was inserted into the test tube then 1 mL of 0.6% (w/v) starch solution was added. The standard solution and the sample were incubated at 50 °C for 10 minutes, then 1 mL of 1 N HCl was added to stop the reaction. Subsequently, 1 mL iodine solution was added and then diluted to 5 mL. The absorbance in each solution was measured at a wavelength of 600 nm using the Genesys 20 Spectrophotometer. The calibration curve was made using the absorbance of the standard solution then a linear equation was obtained based on the resulting absorbance. The absorbance control was used when the starch solution concentration was 6 mg/mL. The value of 10 was based on the amount of amylase, which can cause a 10% decrease in the intensity of the iod-amylase blue under test conditions. The analysis using the Fuwa method was based on a decrease in the intensity of the blue caused by the reaction between iodine and starch. The decrease in blue occurs due to the reduced amount of starch.

Enzymatic hydrolysis process

The hydrolysis method used 20 g of *C. pyrenoidosa*. A sample of 20 g of *C. pyrenoidosa* was placed into a beaker glass and added with 100 mL of aquadest and HCl solution until pH-4.5.

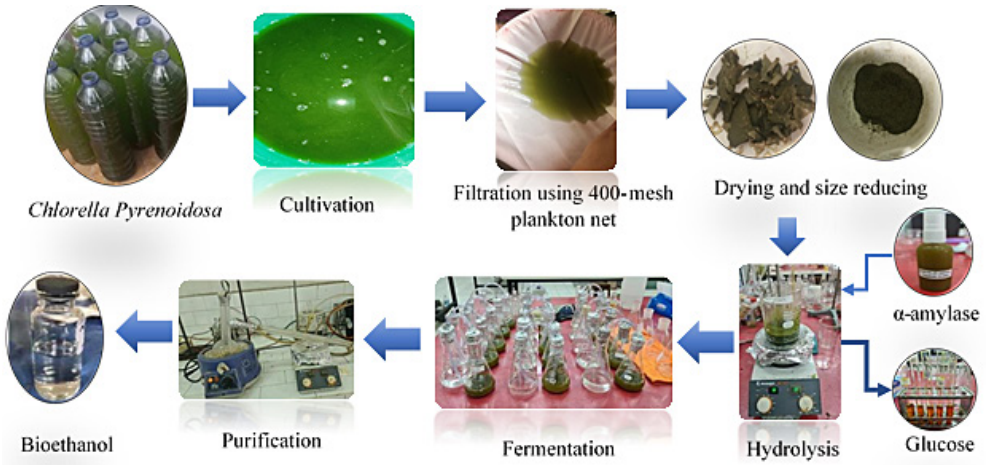

Figure 1. Bioethanol Process from *Chlorella pyrenoidosa*

The acidic condition affected the total amount of protein charge contained in the enzyme and the amino acid load. Amino acids function as a binder to the substrate in the complex sugars and convert them into simple sugars. The hydrolysis process was a slow-running reaction, so a catalyst was needed to speed up the reaction. The hydrolysis process at lower temperatures takes longer to produce large amounts of glucose due to the enzyme performance decreasing and optimal conditions. The catalyst used in this study was the α-amylase enzyme. The hydrolysis process used enzymes that required optimum temperature conditions. The α-amylase enzyme has an optimum temperature of 20–50° (Fitrianti et al., 2014). The enzyme was added to the sample solution at 10%, 20%, 30%, 40%, and 50% in (v/w). The solutions were hydrolyzed at 45 °C for 45 minutes which was the optimum time for enzymatic hydrolysis (Padil et al., 2017) and stirred using a magnetic stirrer at 200 rpm. The speed of stirring in the hydrolysis process can be affected the distribution of the substrate molecules and the number of collisions between the catalyst and the substrate. Slow stirring took a longer time for the hydrolysis process to make the catalyst's chance to react to the substrate became longer. After the reaction, the solutions were separated using a centrifuge. The liquid at the top was taken as a hydrolysis solution. The hydrolysis solution in the amount of 0.2 mL was taken for glucose analysis using the DNS method.

Fermentation process

The hydrolysis solution was transferred to the Erlenmeyer flask, and then 50 mL of 1 N NaOH solution was added until pH-4.8. S. cerevisiae was added to the solution as much as 1% (w/v) of the total volume of the solution. The solution was homogenized using a rotary shaker at a speed of 220 rpm for 1 minute. Erlenmeyer flask is tightly closed to avoid incoming air. Fermentation was carried out for 3, 5, 7, 9, and 11 days at 30 °C.

MATERIALS AND METHODS

The effect of α-amylase concentration on the hydrolysis process

The hydrolysis process in this study was carried out enzymatically using the α-amylase enzyme with various concentrations for each sample (10%, 20%, 30%, 40%, and 50% (v/w)) at 40 °C for an hour. The α-amylase enzyme activity was analyzed by using the Fuwa method before use. The enzyme activity analysis began with the preparation of 0.1 N iodine solution using 1.269 g of iodine solids and 50 mL of K.I. solution added into the Erlenmeyer flask. Then, 50 mL of aquadest was added to the solution, and the K.I. solution could be added again until the iodine solid dissolves. The standard solution of the Fuwa method was prepared in the different Erlenmeyer with various starch 0.2, 0.4, 0.6, 0.8, and 1 g.

Each sample was dissolved in 100 mL aquadest for concentrations of 2, 4, 6, 8, and 1 mg/mL solutions. The standard solution of 1 mL was inserted into the test tube then 1 mL of 0.6% (w/v) starch solution was added. The standard solution and the sample were incubated at 50 °C for 10 minutes, then 1 mL of 1 N HCl was added to stop the reaction. 1 mL iodine solution was added and then diluted to 5 mL. The absorbance in each solution was measured at a wavelength of 600 nm using the Genesys 20 Spectrophotometer. The calibration curve was made using the absorbance of the standard solution then a linear equation was obtained based on the resulting absorbance. The activity of the α-amylase enzyme can be determined using equation 1. The absorbance control was used when the starch solution concentration was 6 mg/mL. The value of 10 was based on the amount of amylase, which can cause a 10% decrease in the intensity of the iod-amylose blue under test conditions.

$$\text{Activity}\left(\frac{\text{Unit}}{\text{mL}}\right) = \frac{A_k - A_s}{A_k} \times fp \text{ x} \frac{1}{Ve} \times 10 \quad (1)$$

where: A_k – the absorbance of the control;
A_s – the absorbance of the sample;
fp – the dilution factor;
V_e – the volume of the enzyme (mL).

The enzyme activity was determined using the enzyme activity equation (1), and based on this study, the number of active enzymes in the sample solution was 32.72 u/mL. Tables 1 and 2 showed that the absorbance of the sample solution was below the absorbance of the standard solution (starch concentration < 2 mg/mL), which meant that the amount of starch remaining in the sample decreased after adding the α-amylase enzyme. The reduced amount of starch after being given the α-amylase enzyme indicates that the

Table 1. The absorbance results of standard solutions in the α-Amylase enzyme activity analysis

Concentration of standard solution (mg/mL)	Absorbance
2	0.313
4	0.350
6	0.463
8	0.531
10	0.645

Table 2. Absorbance results of sample solutions in the α-amylase enzyme activity analysis

Sample solution	Absorbance
S1	0.160
S2	0.160

α-amylase enzyme in the sample has converted a lot of starch into glucose compounds, so that the α-amylase enzyme has been tested was suitable for use in the hydrolysis process of this study. In this process, the amino acid in the α-amylase enzyme bonded carbohydrate molecules and converted them into glucose. The glucose was analyzed by using DNS methods (Figure 2).

Figure 2 shows that the highest glucose content was 0.67 mg/mL produced from the hydrolysis of 20 g of *C. pyrenoidosa* treated using 40% (v/w) of α-amylase enzyme and the glucose contents. The glucose contents produced were directly proportional to the amount of enzyme concentration used. More enzymes were added, making contact between the enzymes and carbohydrates increase. A large amount of glucose indicated that the enzyme activity in the solution was high, so the enzymes worked optimally. For the sample with added 50% (v/w) of the α-amylase enzyme, the glucose produced tended to decrease, because the amount of α-amylase enzyme was too great to hydrolyze carbohydrates in 20 g of *C. pyrenoidosa* and made the number of enzymes unbalanced compared to the number of samples. Using the α-amylase enzyme from *A. niger* as a bio-catalyst in the enzymatic hydrolysis process can reduce energy consumption by around 10–20% (Fang et al., 2019). It was the ideal and natural hydrolysis process to produce glucose from *C. pyrenoidosa*.

Effect of fermentation time on bioethanol contents

The fermentation process in this study reacted to glucose products from the hydrolysis process using *S. cerevisiae* to produce bioethanol and carbon dioxide. This process was conducted at room temperature with pH-4.8 by adding 1 M NaOH to make the solution under acidic conditions. This acid conditioning was carried out to increase the pH of the hydrolysis solution so that the performance of *S. cerevisiae* was optimal because the yeast only endured in acidic conditions. This study aimed to analyze the effect of fermentation on the bioethanol content with various fermentation times (3, 5, 7, 9, and 11) days. The product was analyzed by using GCMS analysis. The highest bioethanol content was obtained from the sample treated with 40% of α-amylase enzyme and fermented for 9 days. Figure 3 shows that the increasing bioethanol contents followed the concentration of the α-amylase enzyme. The sample treated with 50% (v/w) of α-amylase

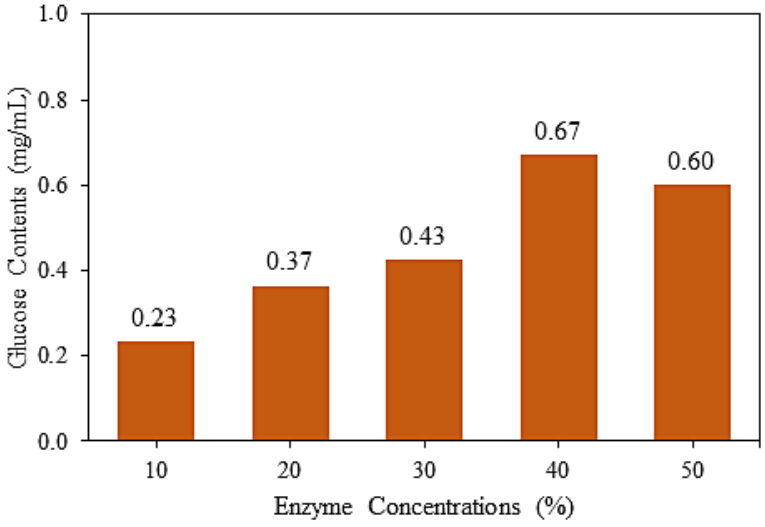

Figure 2. Graphic of calibration of standard glucose solution

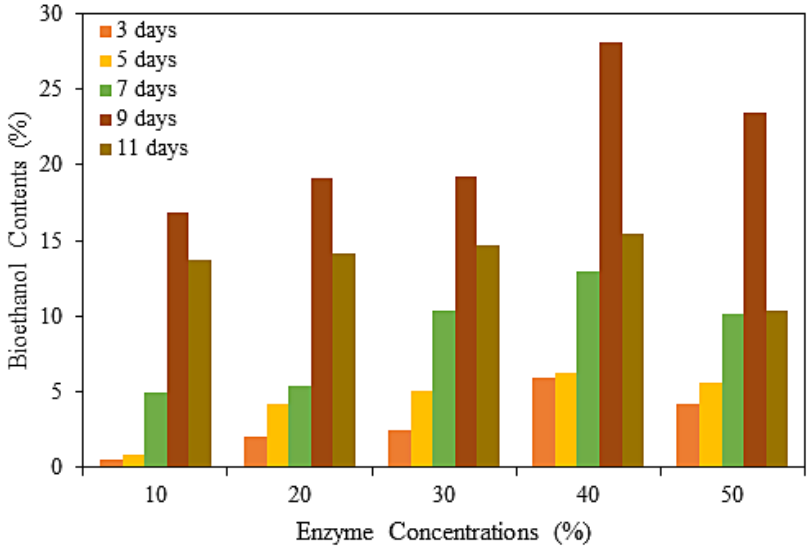

Figure 3. The effect of enzyme concentration and fermentation time on bioethanol contents

enzyme produced less bioethanol content. It occurred because the glucose content produced in the hydrolysis process was also lower.

The optimum fermentation time for *S. cerevisiae* was on the 9th day. Meanwhile, the glucose content in the sample for 11 days decreased. The reduced availability of glucose caused some *S. cerevisiae* to become inactive in 11 days of fermentation. The *S. cerevisiae* active converted glucose to bioethanol in 2 – 4 days (Irvan et al., 2016) due to the yeast growth phase, and it still constantly converted the glucose in 4 – 9 days as its stationary phase. The fermentation occurred for more than 9 days, and yeast activation decreased due to a lack of substrate and nutrition. From this study, one kilogram of *C. pyrenoidosa* as raw material produced 750 mL of bioethanol.

CONCLUSIONS

In this study, the carbohydrate contained in the *C. pyrenoidosa* as raw material was converted effectively into glucose using 40% (v/w) of α-amylase enzyme and analyzed using the DNS method. It produced 0.67 mg/mL of yield glucose, fermented by using *S. cerevisiae* and added some nutrition for 9 days, producing 28.07% of bioethanol content.

REFERENCES

1. Bolivar-Telleria, M., Turbay, C., Favarato, L., Carneiro, T., De Biasi, R.S., Fernandes, A.A.R., Santos, A. M.C., Fernandes, P.M.B., Lei, Z. 2018. Second-Generation Bioethanol from Coconut Husk. BioMed Research International, 2018. https://doi.org/10.1155/2018/4916497
2. Das, S., Singh, S., Sharma, V., Soni, M.L. 2011. Biotechnological applications of industrially important amylase enzyme. International Journal of Pharma and Bio Sciences, 2(1), 486–496.
3. Dhull, N.P., Gupta, K., Soni, S.K. 2014. pyrenoidosa and The Enzymatic Hydrolysis of Its Biomass for The Synthesis of Third Generation. PeerJPrePrint, 1(2014), 50–51.
4. Fang, W., Xue, S., Deng, P., Zhang, X., Wang, X., Xiao, Y., Fang, Z. 2019. AmyZ1: A novel α-amylase from marine bacterium Pontibacillus sp. ZY with high activity toward raw starches. Biotechnology for Biofuels, 12(1), 1–15. https://doi.org/10.1186/s13068-019-1432-9
5. Fitrianti, R., Harianie, L., Ahmad, M. 2014. Pengaruh Suhu dan pH terhadap Aktivitas Enzim Selulase dari Kultur Campuran Trichoderma sp. Gliocladium sp, dan Botrytis sp. yang Ditumbuhkan Pada Media Kulit Pisang. Fisiologi, 61–66.
6. Gallagher, D., Parker, D., Allen, D.J., Tsesmetzis, N. 2018. Dynamic Bacterial and Fungal Microbiomes During Sweet Sorghum Ensiling Impact Bioethanol Production. Bioresource Technology, 264(March), 163–173. https://doi.org/10.1016/j.biortech.2018.05.053
7. Goh, C.S., Lee, K.T. 2010. A Visionary And Conceptual Macroalgae-Based Third-Generation Bioethanol (TGB) Biorefinery in Sabah, Malaysia As An Underlay for Renewable And Sustainable Development. Renewable and Sustainable Energy Reviews, 14(2), 842–848. https://doi.org/10.1016/j.rser.2009.10.001

8. Gupta, K., Chundawat, T.S. 2020. Zinc Oxide Nanoparticles Synthesized Using Fusarium Oxysporum to Enhance Bioethanol Production From Rice-Straw. Biomass and Bioenergy, 143(July), 105840. https://doi.org/10.1016/j.biombioe.2020.105840
9. Hassan, M.K., Chowdhury, R., Ghosh, S., Manna, D., Pappinen, A., Kuittinen, S. 2021. Energy and Environmental Impact Assessment of Indian Rice Straw For The Production of Second-Generation Bioethanol. Sustainable Energy Technologies and Assessments, 47(August), 101546. https://doi.org/10.1016/j.seta.2021.101546
10. Hung, H.C., Adeni, D.S.A., Johnny, Q., Vincent, M. 2018. Production of bioethanol from sago hampas via Simultaneous Saccharification and Fermentation (SSF). Nusantara Bioscience, 10(4), 240–245. https://doi.org/10.13057/nusbiosci/n100407
11. Jiang, D., Hao, M., Fu, J., Liu, K., Yan, X. 2019. Potential Bioethanol Production from Sweet Sorghum on Marginal Land in China. Journal of Cleaner Production, 220, 225–234. https://doi.org/10.1016/j.jclepro.2019.01.294
12. Klasson, K.T., Boone, S.A. 2021. Bioethanol Fermentation of Clarified Sweet Sorghum (Sorghum bicolor (L.) Moench) Syrups Sealed and Stored under Vegetable Oil. Industrial Crops and Products, 163(January), 113330. https://doi.org/10.1016/j.indcrop.2021.113330
13. Lee, Y.G., Jin, Y.S., Cha, Y.L., Seo, J.H. 2017. Bioethanol Production From Cellulosic Hydrolysates By Engineered Industrial Saccharomyces cerevisiae. Bioresource Technology, 228, 355–361. https://doi.org/10.1016/j.biortech.2016.12.042
14. Melikoglu, M., Turkmen, B. 2019. Food waste to energy: Forecasting Turkey's Bioethanol Generation Potential From Wasted Crops and Cereals Till 2030. Sustainable Energy Technologies and Assessments, 36(October), 100553. https://doi.org/10.1016/j.seta.2019.100553
15. Mojsov, K.D. 2016. New and Future Development in Microbial Biotechnology and Bioengineering. Elsevier B.V.
16. Moreira, V.R., Lebron, Y.A.R., Freire, S.J., Santos, L.V.S., Palladino, F., Jacob, R.S. 2019. Biosorption of Copper Ions From Aqueous Solution Using Chlorella pyrenoidosa: Optimization, Equilibrium and Kinetics Studies. Microchemical Journal, 145(October 2018), 119–129. https://doi.org/10.1016/j.microc.2018.10.027
17. Nur, M.M.A. 2014. Potency of Microalgae as Source of Functional Food in Indonesia (Overview). Eksergi, 11(2), 1–6. https://doi.org/https://doi.org/10.31315/e.v11i2.363
18. Olempska-Beer, Z.S., Merker, R.I., Ditto, M.D., DiNovi, M.J. 2006. Food-Processing Enzymes from Recombinant Microorganisms-A Review. Regulatory Toxicology and Pharmacology, 45(2), 144–158. https://doi.org/10.1016/j.yrtph.2006.05.001
19. Padil, P., Syamsiah, S., Hidayat, M., Kasiamdari, R.S. 2017. Kinerja Enzim Ganda Pada Pretreatment Mikroalga Untuk Produksi Bioetanol. Jurnal Bahan Alam Terbarukan, 5(2), 92–100. https://doi.org/10.15294/jbat.v5i2.7564
20. Pandebesie, E.S., Kartini, A.M., Wilujeng, S.A., Warmadewanthi, I. 2019. Utilization of Stalks Waste of Sorghum to Produce Bioethanol by Using Saccharomyces cerevisiae and S. cerevisiae-Pichia stipitis Consortium. Journal of Ecological Engineering, 20(4), 54–60. https://doi.org/https://doi.org/10.12911/22998993/102616
21. Raheem, A., Prinsen, P., Vuppaladadiyam, A.K., Zhao, M., Luque, R. 2018. A review on Sustainable Microalgae Based Biofuel and Bioenergy Production: Recent Developments. Journal of Cleaner Production, 181, 42–59. https://doi.org/10.1016/j.jclepro.2018.01.125
22. Rijal, M. 2020. Bioethanol from Sago Waste Fermented by Baker's and Tapai Yeast as a Renewable Energy Source. International Journal of Scientific and Technology Research, 9(3), 4918–4924.
23. Rizzolo, J.A., Woiciechowski, A.L., Júnior, A.I.M., Torres, L.A.Z., Soccol, C.R. 2021. The potential of sweet potato biorefinery and development of alternative uses. SN Applied Sciences, 3(3), 1–9. https://doi.org/10.1007/s42452-021-04369-y
24. Suhartini, S., Rohma, N.A., Mardawati, E., Kasbawati, Hidayat, N., Melville, L. 2022. Biorefining of Oil Palm Empty Fruit Bunches for Bioethanol and Xylitol Production in Indonesia: A Review. Renewable and Sustainable Energy Reviews, 154(December 2020), 111817. https://doi.org/10.1016/j.rser.2021.111817
25. Tan, I.S., Lam, M.K., Foo, H.C.Y., Lim, S., Lee, K.T. 2020. Advances of Macroalgae Biomass For The Third Generation Of Bioethanol Production. Chinese Journal of Chemical Engineering, 28(2), 502–517. https://doi.org/10.1016/j.cjche.2019.05.012
26. Tolulope Eunice, K. 2021. Enzymatic and Dilute Acid Hydrolyses of Maize Stalk Substrate in Bioethanol Production. Journal of Energy, Environmental & Chemical Engineering, 6(1), 24. https://doi.org/10.11648/j.jeece.20210601.14
27. Vitus, J., Otaraku, I.J. 2020. Bioethanol Production from Sweet Potatoes. International Journal of Scientific & Engineering Research, 11(3), 1045–1050.
28. Zhao, X.C., Tan, X.B., Yang, L.B., Liao, J.Y., Li, X.Y. 2019. Cultivation of Chlorella pyrenoidosa in Anaerobic Wastewater: The Coupled Effects of Ammonium, Temperature and pH Conditions on Lipids Compositions. Bioresource Technology, 284(March), 90–97. https://doi.org/10.1016/j.biortech.2019.03.117