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The Ability of the Thermophilic Bacteria Triculture Consortium from Mudiak Sapan Hot Springs to Produce Biofuel

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Abstract: Biofuel is an alternative energy to replace fossil fuels. The most popular form of biofuel today is bioethanol. Biofuels are considered as a suitable alternative to fossil fuels because they are more environmentally friendly. Production of bioethanol utilizing thermophilic microoganisms is more profitable because thermophilic microbes generally have the characteristics of being able to use a variety of substrates, low contamination, and resistance to high temperatures. The use of microbial consortia tends to give better results than the use of monoculture isolates to optimize bioethanol yields. The aim of this study was to determine the compatibility and ability of the best thermophilic bacterial triculture consortium from the Mudiak Sapan hot springs in producing biofuel. This research is a descriptive research, to test isolates of a consortium of thermophilic bacteria that produce bioethanol. The results of this study showed that consortium isolates tended to give better results than monoculture isolates. The best thermophilic bacterial triculture consortium from the Mudiak Sapan hot springs in producing biofuels namely MS 12, 17, 18 produced a bioethanol content of 0.863%.

Keywords: Bioethanol; Biofuels; Compatibility; Consortium; Thermophilic

Introduction

Energy is needed for human activities, especially for economic, household, industrial, business and transportation activities. Most of the energy supply in the world comes from fossil fuels which are non-renewable resources. Energy needs are expected to continue to increase, while the reserves of petroleum and coal are dwindling. The use of fossil fuels as energy contributes to excess carbon in the atmosphere, causing global warming, so there is a need for a supply of alternative energy other than petroleum and coal (Setyono, 2019).

Fossil fuels have been used as the main source of energy for many years, but their use is not renewable and creates environmental problems. This challenge makes it possible to replace fossil fuels with environmentally friendly renewable energy sources such as biofuels. Biofuels (Biofuels) are energy produced by biological processes from the biomass of organisms such as bacteria, microalgae, and plants (Radionova et al., 2017). The most popular forms of biofuels today are bioethanol and biodiesel (Devita, 2015).

Biofuels are produced directly from plants and microorganisms. Biofuels can be divided into three generations. The first generation of biofuels was the production of ethanol from starch of food crops such as wheat, barley. The second generation of biofuels is the production of bioethanol and biodiesel from several types of plant wastes such as straw, grass and wood. The third generation of biofuels is the production of bioethanol from microalgae and microorganisms (Rodionova et al., 2017). Thermophilic microorganisms for bioethanol production include bacteria and fungi that are most widely used to produce bioethanol, namely thermophilic bacteria (Riyanti, 2011).

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Thermophilic microorganisms are microorganisms that can live at temperatures of 45oC–88oC. Thermophilic microorganisms contain heat-resistant and denaturation-resistant proteins so they can adapt to extreme temperature environmental conditions (Firliani et al., 2015). One of the advantages of thermophilic microorganisms in the industrial world is the production of bioethanol (Martosuyono & Rogers., 2005).

The bioethanol production process that utilizes thermophilic microorganisms has several advantages. Thermophilic microbes generally have the characteristic of being able to use a variety of substrates, thermophilic bacteria are able to survive and develop in high temperature conditions because the proteins of thermophilic bacteria are more stable and heat resistant compared to mesophilic bacteria (Mawati et al., 2021). High temperature bioprocess offers a reduced risk of contamination from unwanted microorganisms. Types of thermophilic bacteria that produce bioethanol Baccilus sp. , Clostridium sp. (Geraldi et al., 2019).

Thermophilic bacteria can be found in various places in nature, such as hot springs, areas of volcanic activity, or on the seabed which has hot springs. One of the geothermal springs found in West Sumatra is the Mudiak Sapan hot spring, Jorong Balun, Nagari Pakan Rabaa, Koto Parik Gadang District in Ateh. This hot spring has a temperature of 93oC with a pH of 8 (Irdawati et al., 2016).

Microorganisms found in nature are not only in single form but in mixtures. The consortium is a mixture of microbial populations in the form of communities that have cooperative, commensal, and mutualistic relationships. Community members who have a relationship will associate. The relationship between consortium bacteria in sufficient substrate conditions will not interfere with each other, but synergize with each other so as to produce higher decomposition efficiency during the processing. The bacterial culture used as a consortium must be compatible (Asri & Zulaika, 2016).

Bacterial compatibility is an association between two genera or certain species of bacteria that do not interfere with each other, but the activities of each genus or species are mutually beneficial. The compatibility or synergism of two or more inoculated bacteria is a very important factor so that these bacteria can work together well (Asri & Zulaika, 2016). The bacterial consortium is said to be compatible if there is no zone of inhibition between the bacterial isolates (Fitriasari et al., 2020).

The use of a microbial consortium tends to give better results than the use of a single isolate, because it is expected that the enzyme work of each type of microbe can complement each other in order to survive using the available nutrient sources in the carrier media (Asri & Zulaika, 2016). According to Donato et al., (2019) Clostridium thermocellum and Clostridium thermolacticum co-cultures produced higher bioethanol, which was 0.53% compared to Clostridium thermocellum monoculture, which was 0.38%. In a study by Vinotha & Umamaheswari (2019) reported that a consortium of Pseudomonas aeroginosa and Bacillus clausii bacteria produced 1.32% -1.44% ethanol.

Research conducted by Agusri (2022) used a single isolate with the thermophilic bacteria MS (Mudiak Sapan), namely 12 isolates produced the four highest isolates including MS-9, MS-12, MS-18, MS-17. The highest yield was MS-9 which produced 1,001% bioethanol. The four isolates were used in this study as selected isolates for the consortium's bioethanol production. The use of a microbial consortium is expected to provide better results than the use of a single isolate, because the action of enzymes from each type of microbe can complement each other (Asri & Zulaika, 2016). Based on the background that has been described, the researchers decided to conduct research on "The Ability of the Thermophilic Bacteria Triculture Consortium from Mudiak Sapan Hot Springs to Produce Biofuel".

Method

This research is a descriptive study. To test the isolates of the thermophilic bacteria producing bioethanol, a compatibility test was carried out using the disk diffusion method, then the consortium isolates were fermented with liquid TMM (Thermophilic Minimum Media) medium and the bioethanol content was measured using a distillation apparatus.

Preparation of Liquid TMM Medium

The medium for growing bioethanol-producing thermophilic bacteria is using liquid TMM (Thermophilic Minimum Media) medium with a composition of 0.01% MgSO4.7H2O, 0.1% K2HPO4, 0.35% (NH4)2SO4, 0.1% NaCl, 0. 05% yeast extract, 0.05% peptone, 6% glucose (Zilda et al., 2008). TMM Dissolved with distilled water up to 1000 ml then heated until homogeneous and then sterilized in an autoclave with a temperature of 121°C at a pressure of 15 psi for 15 minutes.

Compatibility Test

Mudiak Sapan (MS) Isolate compatibility test using the disk diffusion method. The compatibility test of thermophilic bacterial isolates MS 9 & MS 4 was carried out by taking 5 oses of MS 9, then putting them into a test tube containing 5 ml of sterile distilled water and adjusting the population density to a scale of 1 Mc. Farland's (population 3x108 cells/mL). 1 mL of isolate suspension (1 McFarland's scale) was put into a sterile petri dish. Then it is poured with NA medium, homogenized by rotating the petri dish like a figure eight and allowed to cool down. Next, 4 sheets of sterile disc paper were taken, placed in a sterile petri dish and then dripped with 0.1 mL of isolate suspension and allowed to stand for a while. Then the disc was placed in the middle of the medium which had been inoculated with the isolate suspension and incubated for 2 x 24 hours at 50oC. The same procedure was carried out for the other combinations of isolates. Compatible isolates were indicated by the absence of an inhibition zone formed, while isolates that were not compatible were indicated by the presence of an inhibition zone formed (Jovanita et al., 2022).

Production of Triculture Consortium Isolates

Making consortium isolates with 4 isolates of thermophilic bacteria MS 9, MS 12, MS 18, and MS 17 isolates were taken as much as 5 ose each from the slanting agar and put into a test tube containing 5 ml of 0.85% NaCl to the equivalent of McFarland scale of 0.5. Then 2.5 ml of the bacterial suspension was put into an Erlenmeyer containing 22.5 ml of liquid TMM medium, then incubated for 24 hours to be activated in an incubator at 60oC. After 24 hours, 0.25 ml of activation medium was taken and put into a test tube containing 5 ml of physiological salt (0.85% NaCl) and compared with 0.5 of Mc Farland's solution. Suspension was taken as much as 10 ml to make a triculture consortium (MS-9, MS- 17, MS- 18), (MS- 9, MS- 12, MS- 18), (MS- 9, MS-12, MS- 17) , (MS- 12, MS- 17, MS- 18) with a ratio of 1:1:1 was put into 40 ml of liquid TMM and then incubated for 24 hours in an incubator with a temperature of 60oC, pH 8. After completion of fermentation, distillation was carried out with a pycnometer to measure bioethanol (Vinota et al., 2019; Safari & Syafaat, 2022).

Result and Discussion

Thermophilic Bacteria Consortium Compatibility Test

Based on research on the compatibility test of the consortium of thermophilic bacteria in producing biofuels, it was found that all the isolates of the consortium synergized with one another, this is indicated by the absence of clear zones, as shown in table & Figure 1.

In table 1. It can be seen that the MS 9 & MS 18 consortium onwards have good consortium capabilities and can produce bioethanol. This is what underlies the selection of a consortium of thermophilic bacteria that produce bioethanol.

The compatibility test of the thermophilic bacteria of the Mudiak Sapan hot springs was carried out with the aim of obtaining consortium isolates that synergize with one another and have the ability to produce bioethanol. The combination of the bacterial consortium uses a combination of four isolates that have the ability to produce bioethanol selected based on the combination. Incubation was carried out for 2 x 24 hours and observed the formation of inhibition zones.

Table 1. The results of the MS isolate combination compatibility test

Test Isolate	Compatibility	Results
MS 9 & MS 18	+	Compatible
MS 9 & MS 12	+	Compatible
MS 9 & MS 17	+	Compatible
MS12&MS 17	+	Compatible
MS12&MS 18	+	Compatible
MS17&MS 18	+	Compatible

Information:
(+): Compatible
(-): Non Compatible

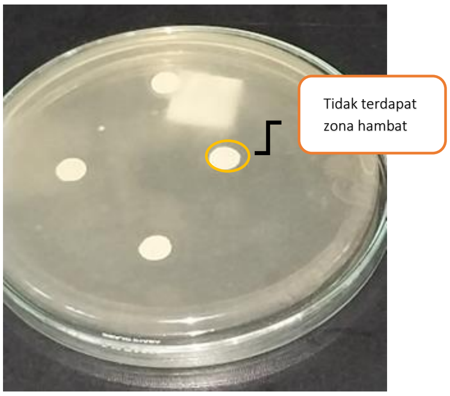

Figure 1. Bacterial Consortium Compatibility Test

According to Asri & Zulaika, (2016) bacterial compatibility is an association between two genera or species of certain bacteria that do not interfere with each other, but the activities of each genus or species are mutually beneficial, and share the same nutritional sources in different living media. The same. The bacterial cultures used as a consortium must be compatible. The existence of compatibility or synergism of two or more inoculated bacteria is a very important factor so that these bacteria can work together well.

The mechanism of synergism between isolates in the consortium is caused by several factors, including: (1) one member of the genus is able to provide one or more nutritional factors that cannot be synthesized by other members of the genus, (2) one member of the genus is unable to degrade organic matter certain species will depend on the members of the genus that are able to provide the results of the degradation of the organic matter, (3) one member of the genus protects other members of the genus that are sensitive to certain organic matter by reducing the concentration of toxic organic matter by producing specific or non-specific protective factors (Deng & Wang, 2016).

Thermophilic Bacteria Triculture Consortium Fermentation in Bioethanol Production

Research on thermophilic bacterial triculture consortium fermentation in bioethanol production can be seen in (Table 2). Triculture consortium isolates of thermophilic bacteria in the highest bioethanol production were MS 9, 12, 17 with 1.006% bioethanol content followed by consortium isolates MS 12, 17, 18 with 0.863% bioethanol content which was higher than the monoculture isolates. Whereas in the fermentation of monoculture isolates the lowest was MS 12 with a bioethanol content of 0.173%.

Tabel 2. Bioethanol Content from Triculture Consortium Isolates

Bacterial Consortium Isolate	Bioethanol Content (%)
MS 9	1.002
MS 12	0.173
MS 17	0.518
MS 18	0.630
MS 9, 12, 18	0.203
MS 9, 17, 18	0.448
MS 9, 12, 17	1.006
MS 12,17,18	0.863

The results of research regarding the fermentation of thermophilic bacterial triculture consortium in bioethanol production are based on (Table 3). Triculture consortium isolates MS 9, 12, 17 produced the highest average value of bioethanol content, namely 1.006% and isolate MS 12 produced the lowest bioethanol content, namely 0.173%. In accordance with the statement of Asri & Zulaika., (2016) that consortium isolates are able to provide better results than monoculture isolates, because the action of enzymes from each type of microbe can complement each other. It can be seen that the synergism of MS 9, 12, 17 is better than other MS isolates because of the optimal physiological cooperation of the three isolates resulting in higher bioethanol.

The triculture consortium isolates MS 9, 12, 17 produced higher bioethanol levels than the monoculture isolates with 1.006% bioethanol content, as well as MS isolates 12,17,18. Meanwhile, MS isolates 9, 12, 18 produced 0.203% bioethanol content because MS 12, which had a relatively low bioethanol content, likely affected the other 2 isolates, resulting in lower bioethanol levels compared to other triculture consortium isolates. The most successful bioethanol fermentation in thermophilic bacterial tricultures were isolates MS 12, 17, 18 with a bioethanol content of 0.863% because they significantly increased the production of bioethanol compared to monoculture isolates, after consortium they produced two times higher levels of bioethanol.

Microbes in the consortium have a great opportunity to gain energy and survive, because they can mutually utilize coenzymes excreted by other microbes, besides that other microbes can decompose substrates that have been previously degraded by a microbe (Septiningrum, 2011). Firdaus (2018) described several advantages of using a microbial consortium. Among them are being able to carry out sequential degradation, the consortium being able to produce the enzymes or substances needed, being able to increase the overall rate of substrate degradation, being able to facilitate oxidation, because it can find the easiest thermodynamic pathway.

In Table 2. it can be seen that the monoculture isolate MS 12 has the lowest bioethanol content. This is because the monoculture isolate is not optimal in degrading chemical compounds in bioethanol production. Jovani & Advinda (2022) emphasized that consortium members who have relationships will associate, so they are more successful in degrading chemical compounds than monoculture isolates. According to research conducted by Pandebesie & Kartini (2016) the S. cerevisiae-P. Stypitis produced a higher ethanol content of 2.1% at the 24th hour of fermentation. Compared with monoculture S. cerevisiae 0.725%. In a study by Donato et al. (2019) co-cultures of Clostridium thermocellum and Clostridium thermolacticum produced higher bioethanol, namely 0.53%, compared to monocultures of Clostridium thermocellum, which was 0.38%. In Liu et al.'s study, (2017) co-culture of Aspergillus niger, Trichoderma reesei, Zymomonas mobilis produced a bioethanol content of 50%.

According to Bagaskara et al. (2020) bioethanol can be produced through a fermentation process that utilizes the help of microorganisms. Microorganisms that are widely used in the fermentation process are yeast and bacteria. Currently, Saccharomyces cerevisiae from the yeast group is used as an ethanol-producing microorganism. However, Saccharomyces cerevisiae has several drawbacks, including high biomass production and inability to produce high concentrations of ethanol. While thermophilic bacteria have many properties that make them suitable for bioethanol production. Donato et al. (2019), suggested that thermophilic bacteria are not only able to efficiently degrade cellulose and hemicellulose, but can easily ferment pentose and hexose sugars produced after polysaccharide hydrolysis to produce bioethanol. Thermophilic bacteria are able to survive and develop in high temperature conditions because the proteins of thermophilic bacteria are more stable and heat resistant, as well as lower contamination (Mawati et al., 2021).

According to Albert et al., (2015) The basic principle of fermentation is activating certain microbial activities with the aim of changing the properties of the material to produce a useful material. This change is due to the fact that in the fermentation process the number of microbes is increased and their metabolism in the material is activated within certain limits. the longer the fermentation, the lower the glucose level and the higher the alcohol content because during fermentation the glucose contained in the substrate (ingredient) will be converted by enzymes into alcohol and carbon dioxide (CO2).

Based on research by Martosuyono & Rogers (2005) to test isolates of thermophilic bacteria that produce bioethanol grown on solid selective medium TMM (Thermophilic Minimum Media). In addition, TMM can also be used for fermentation medium with the same composition without using bakto agar. The composition of the TMM medium contained 6% glucose, where glucose was used as a carbon source for the formation of ethanol in this study. The process of decomposing sugar by microbial activity in which the chemical bonds of the carbon chains of glucose and fructose are released one by one and chemically assembled into ethanol molecules and carbon dioxide gas and produces heat. The reaction for the formation of ethanol from glucose is as follows:

$C_6H_{12}O_6 \rightarrow$ 2C2H5OH + 2CO2 + less energy and in an anaerobic atmosphere.

Conclusion

The thermophilic bacteria consortium isolates from Mudiak Sapan hot springs are compatible. The best thermophilic bacterial triculture consortium from Mudiak Sapan hot springs in producing biofuels, namely MS 12, 17, 18 with a ratio of 1: 1: 1 produced a bioethanol content of 0.863%.

References

Agusri, R. (2022). *Optimasi Suhu Bakteri Termofilik dalam Menghasilkan Bioetanol Sebagai Biofuel.* Skripsi, Universitas Negeri Padang. Retrieved from http://repository.unp.ac.id/39806/

Albert, Idiawati, N., & Rudiyansyah (2015). Pembuatan Bioetanol Mengunakan Zymomonas Mobilis Dari Limbah Tongkol Jagung. *Jurnal Kimia Khatulistiwa*, *4*(2). Retrieved from https://jurnal.untan.ac.id/index.php/jkkmipa/article/view/11736

Asri, A.C. and Zulaika, E., (2016). Sinergisme antar isolat Azotobacter yang dikonsorsiumkan. *Jurnal sains dan seni ITS*, *5*(2). http://dx.doi.org/10.12962/j23373520.v5i2.20693

Bagaskara, A., Wijaya, I. M. M., & Antara, N. S. (2020). Isolasi dan karakterisasi bakteri penghasil bioetanol dari lingkungan industri arak di Desa Tri Eka Buana, Kecamatan Sidemen, Karangasem Bali. *Jurnal Rekayasa dan Manajemen Agroindustri, 8*(2), 290-300. https://doi.org/10.24843/JRMA.2020.v08.i02.p13

Deng, Y. J., & Wang, S. Y. (2016). Synergistic growth in bacteria depends on substrate complexity. *Journal of Microbiology*, *54*(1), 23-30. https://doi.org/10.1007/s12275-016-5461-9

Devita, L. (2015). Biodiesel sebagai bioenergi alternatif dan prospeftif. *Agrica Ekstensia*, *9*(2), 23-26. Retrieved from https://www.polbangtanmedan.ac.id/pdf/Jurnal%202015/Vol%209%20No%202/04%20LIZA.pdf

Donato, P., Finore, I., Poli, A., Nicolaus, B. and Lama, L. (2019). The production of second generation bioethanol: The biotechnology potential of thermophilic bacteria. *Journal of cleaner production*, *233*, pp.1410-1417. https://doi.org/10.1016/j.jclepro.2019.06.152

Firdaus, N. Q. (2018). *Efektivitas variasi inokulan konsorsium mikroorganisme (phanerochaete chysosporium, basillus circulans, tricoderma reesei, dan saccharomyces cerevisiae) dalam proses biodelignifikasi rumput gajah (pannisetum Purpureum) dengan penambahan urea*. Bachelor's thesis. Jakarta: Fakultas Sains dan Teknologi Universitas Islam Negeri Syarif Hidayatullah Jakarta. Retrieved from https://repository.uinjkt.ac.id/dspace/handle/123456789/47857

Firliani, W., Agustien, A., & Febria, F. A. (2015). Karakterisasi Bakteri Termofilik Penghasil Enzim Protease Netral. *Jurnal Biologi UNAND*, *4*(1). Retrieved from http://jbioua.fmipa.unand.ac.id/index.php/jbioua/article/view/112

Fitriasari, P. D., Amalia, N., & Farkhiyah, S. (2020). Isolasi dan uji kompatibilitas bakteri hidrolitik dari tanah tempat pemrosesan akhir Talangagung, Kabupaten Malang. Berita Biologi, 19(1), 151-156. Retrieved from https://e-journal.biologi.lipi.go.id/index.php/berita_biologi/article/view/3828

Geraldi, A., Wanguyun, A.P. and Hariyanto, S. (2019). Bio Prospecting Thermostable Enzymes-Producing Thermophiles from Indonesia. *Ecology, Environment and Conservation*, *25*(2019), S75-S79. Retrieved from http://www.envirobiotechjournals.com/article_abstract.php?aid=9690&iid=276&jid=3

Irdawati, I., Fifendy, M., & Ningsih, Y. (2015). Pengaruh Ragi dari Daerah Berbeda Terhadap Kadar Glukosa dan Alkohol Serta Nilai Organoleptik Tapai Ubi Jalar Merah (*Ipomoea batatas* L.). *EKSAKTA*, 2, 42. Retrieved from https://ejournal.unp.ac.id/index.php/eksakta/article/view/5761

Irdawati, I., Syamsuardi, S., Agustien, A., & Alberida, H. (2016). *Profil Pertumbuhan Bakteri Termofilik Penghasil Xylanase Alkali dari Sumber Air Panas Mudiak Sapan, Solok Selatan*. Universitas Negeri Padang. Retrieved from http://repository.unp.ac.id/27774/

Jovanita, L., & Advinda, L. (2022). Compatibility Test of Fluorescent Pseudomonad Isolated from Plant Rhizosphere. *Jurnal Serambi Biologi, 7(*1), 65-69. Retrieved from https://serambibiologi.ppj.unp.ac.id/index.php/srmb/article/view/21

Liu, Y. K., Chen, W. C., Huang, Y. C., Chang, Y. K., Chu, I. M., Tsai, S. L., & Wei, Y. H. (2017). Production of bioethanol from Napier grass via simultaneous saccharification and co-fermentation in a modified bioreactor. *Journal of bioscience and bioengineering*, *124*(2), 184-188. https://doi.org/10.1016/j.jbiosc.2017.02.018

Martosuyono, P., & Rogers, P. L. (2005). Stabilitas Panas Enzim PDC Dari Bakteri Termofil Penghasil Etanol. *Jurnal Penelitian Pascapanen Pertanian*, *2*(2), 49-55. Retrieved from https://repository.pertanian.go.id/items/6d3212d1-d121-4204-a08f-559e69c24a17

Mawati, S. D., Harpen, E., & Fidyandini, H. P. (2021). Skrining Bakteri Termofilik Potensial Amilolitik Dari Sumber Air Panas Way Belerang Kalianda Lampung Selatan. *Journal of Aquatropica Asia*, *6*(1), 1-7. https://doi.org/10.33019/aquatropica.v6i1.2458

Pandebesie, E. S., & Kartini, A. M. (2016). PRODUKSI BIOETANOL dari BATANG Sorghum bicolor (L.) Moench dengan Saccharomyces cerevisiae dan KONSORSIUM S. cerevisiae-Pichia stipitis. *Jurnal Purifikasi*, *16*(2). https://doi.org/10.12962/j25983806.v16.i2.43

Riyanti, E. I. (2011). Beberapa Gen pada Bakteri yang Bertanggung Jawab terhadap Produksi Bioetanol. *Jurnal Litbang Pertanian*, *30*(2), 23-25. Retrieved from https://core.ac.uk/reader/347647340

Rodionova, M.V., Poudyal, R.S., Tiwari, I., Voloshin, R.A., Zharmukhamedov, S.K., Nam, H.G., Zayadan, B.K., Bruce, B.D., Hou, H.J. and Allakhverdiev, S.I. (2017). Biofuel Production: Challenges and Opportunities. *International Journal of Hydrogen Energy*, *42*(12), 8450-8461. https://doi.org/10.1016/j.ijhydene.2016.11.125

Safari, W. F., & Syafaat, M. (2022). Effect of Pretreatment and Composition of Trichoderma Viride and Zymomonas Mobilis Consortium on Bioethanol Production from Leaf Litter. *Indonesian Journal of Biotechnology and Biodiversity*, *6*(2), 52-60. https://doi.org/10.47007/ijobb.v6i2.134

Septiningrum, K. (2011). Produksi xilanase dari tongkol jagung dengan sistem bioproses menggunakan bacillus circulans untuk pra-pemutihan pulp. *Journal of Industrial Research (Jurnal Riset Industri), 5*(1). Retrieved from http://litbang.kemenperin.go.id/jriXX/article/view/84/0

Setyono, J., Mardiansjah, FH., Astuti, MFK. (2019). Potensi Pengembangan Energi Baru dan Energi Terbarukan di Kota Semarang. *Jurnal Riptek, 13*(2) 177-186. Retrieved from https://riptek.semarangkota.go.id/index.php/riptek/article/view/68

Vinotha, T., & Umamaheswari, N. (2019). Bioethanol Production Using Bacterial Consortia from Waste Cellulosic Waste. *Think India Journal*, *22*(10), 4512-4521. Retrieved from https://thinkindiaquarterly.org/index.php/think-india/article/view/11440

Zilda, D. S., Kusumarini, A., Chasanah, E. (2008). Penapisan dan Karakterisasi Protease dari Bakteri Termo-Asidofilik P5-A dari Sumber Air Panas Tambarana. *Jurnal Pascapanen dan Bioteknologi Kelautan dan Perikanan, 3*(2), 113–121. http://dx.doi.org/10.15578/jpbkp.v3i2.17