Biorefinery of genetically modified soybean as biodiesel with sustainable production system in Indonesia: A review

Irfan Anwar Fauzan\textsuperscript{1}, Erliza Hambali\textsuperscript{2,3}

\textsuperscript{1}Biotechnology, IPB University, Jl. Raya Dramaga, Babakan, Dramaga, Bogor 16680, Indonesia
\textsuperscript{2}Department of Agroindustrial Technology, Faculty of Agricultural Technology, IPB University, Jl. Raya Dramaga, Babakan, Dramaga, Bogor 16680, Indonesia
\textsuperscript{3}Surfactant and Bioenergy Research Center, IPB University, Baranangsiang Campus, Bogor, Indonesia

*Email: anwarfauzan@apps.ipb.ac.id

\textbf{Abstract.} Fossil energy consumption in Indonesia is high every year, and this can cause an imbalance between demand and the availability of available fuel. Besides, fossil fuels are also classified as non-renewable fuels, so that the availability of fuel can be used up if exploited continuously. High diesel consumption makes it necessary to search for other energy sources that can be used to meet diesel needs in Indonesia. Alternative diesel replacement that can be used is biodiesel fuel from soybeans. One of the disadvantages of using soy as a biodiesel feedstock is its low oil content. An alternative that can be done is by using genetically modified (GM) soybeans that have higher oil content. The biorefinery approach can also be carried out as an effort to make biodiesel production sustainable, both economically and environmentally. GM soybean can be assembled through several stages, which are selection of varieties, assembly of GM soybean, and then testing and licensing. After that, soybeans can be used and utilized in the Indonesian market. The biodiesel production system from GM soybeans using a biorefinery approach is carried out by utilizing residues during production into other products so that it can be an additional income, and reduce the impact of environmental pollution. A high net energy balance ratio (NER) in some biodiesel production from soybeans makes biodiesel production from soybeans can be said to be renewable and economically sustainable. Biodiesel production from GM soybeans with the concept of biorefinery has the opportunity to become a sustainable industry, both economically and environmentally.

1. \textbf{Introduction}

Energy is an important thing for the people of Indonesia today. The energy that is widely used by the people of Indonesia, both in the form of electrical energy or energy in the form of oil using raw materials classified as fossil fuels. Based on USDA data in 2018, Indonesia's consumption of fossil fuels increased from 58.51 million kL in 2009 to 67 million kL in 2017 \cite{1}. The high use of fossil fuels can cause an imbalance between demand and the availability of fuel which exists. Besides, fossil fuels are also classified as non-renewable fuels, so the availability of fuel can be used up if exploited continuously. This high consumption makes Indonesia must export fossil fuels to meet domestic needs since 2006. USDA in 2017 predicts that fossil energy consumption in Indonesia in 2027 will increase by approximately 46% from consumption in 2017 \cite{1}.
Based on projections from the USDA 2017 in 2027, diesel is one type of fuel that will be consumed a lot. Diesel consumption in 2027 is estimated to reach 57 million kL. High diesel consumption makes it necessary to search for other energy sources that can be used to meet diesel needs in Indonesia. One alternative to substituting diesel is by using biodiesel fuel. Biodiesel is a clean and renewable energy source [2]. Biodiesel is one of the fuels that has begun to be developed in Indonesia. According to PASPI, by replacing diesel with biodiesel in Indonesia, the Indonesian government can save foreign exchange of the country of Rp. 38.88 trillion are obtained. Also, the use of biodiesel saves environmental CO₂ emissions by 11.68 million tons of CO₂ [1].

Biodiesel can be obtained from oil contained in fruit. One of the abundant biodiesel raw materials in Indonesia is soybean. Soybean has the potential to be used as a source of biodiesel because it contains soybean oil. The advantage of using soy as a source of biodiesel in Indonesia is that this plant has been widely planted in almost all of Indonesia, and more when compared to other biodiesel oil-producing plants such as Jatropha curcas and palm oil. Based on this, the availability of soybeans as biodiesel raw material can be more than jatropha and palm oil [3]. The disadvantage of using soybean as a source of biodiesel is that the soybean oil content is low. The percentage of oil content in soybeans is only 20% [4]. One effort that can be done to increase the oil content of soybeans is by genetic engineering methods to make genetically modified (GM) soybean. Modifying fatty acid biosynthesis in soybean to change the amount of relative fatty acids or producing novels of fatty acid compounds in soybean can be done through the soybean engineering process [5,6]. Besides the small amount of oil, another problem is that the production of biodiesel from soybeans in Indonesia must also be sustainable.

Efforts that can be made to make biodiesel production from GM soybeans are sustainable are the biorefinery approach. The biorefinery is an integration of various biomass conversion processes to produce energy and produce other products as value-added products in one system [7]. Biorefinery can be done as an effort to reduce the negative impact of an industry on the environment. This approach can also be carried out as an effort to increase the added value of the product to get benefit economically. Industries that are carried out using the biorefinery approach can be said sustainable Industries [8]. Biodiesel production from GM soybeans can be done with a biorefinery approach as an effort to increase profits, both economically and environmentally. This paper will review the biorefinery approach taken in the biodiesel production industry from GM soybean plants.

2. Discussion

2.1. Assembling GM Soybean Plants

The use of genetically modified (GM) soybean products can be one of the efforts to increase the efficiency of biodiesel production. This is because GM soybean can have higher oil content than other soybeans. Several steps can be carried out to assemble GM soybean plants with high oil content, from research to release to the market. The steps can be seen in Figure 1.
Figure 1. Stages that can be passed to assemble and release GM soybeans to the market in Indonesia

The first step is selecting the soybean varieties to be modified. The number of soybean varieties cultivated in Indonesia currently reaches 73 varieties, some of which have the potential as raw material for making biodiesel. Soybean varieties which used to modified into GM soybean are soybeans that have certain superior characteristics compared to other soybeans. One of the superior traits is having a high resistance to salinity stress. Based on the research of Hakusi in 2017, several local soybean varieties that can grow in saline conditions without experiencing a significant reduction in production yields, which are Karat 13, Grayak 3, Grayak 5, and MLGG 0160 varieties [9]. Besides, many soybean varieties originating from abroad have a tolerance on salinity, such as Lee, Manokin, and Centennial varieties [10].

Other beneficial traits that can be considered are pest resistance, such as armyworm (Spodoptera litura F.). Based on the results of Widyasakta research in 2018, Den 1 soybean varieties have resistance to armyworm attacks based on the anatomical character of the leaves [11]. Soybean productivity is also a matter that can be considered in choosing. Varieties that have high productivity can be chosen because it has the potential to produce more crops. Some superior soybean varieties that have high production rates (> 2 tons / ha) include Baluran (2.5–3.5 t / ha), Merubetiri (2.5–3.0 t / ha), Anjasomoro (2.03–2.25 t / ha) and Mahameru (2.04–2.16 t / ha) [12,13].

The next stage after the selection of soybean varieties is modified the selected varieties genetically. Genetic engineering is carried out to increase the amount of oil contained in soybeans. Oils derived from grains, including soy, are mostly triglycerides, which are compounds that can be processed into biodiesel. Soybean oil also contains other compounds such as phospholipids and tocopherols. Triglycerides contain three chains of fatty acid bonds that are bound to the glycerol backbone. Engineering that can be done to increase the fat content in soybeans can be in the form of increased fatty acid synthesis or increasing the affinity of fatty acids with glycerol backbone. Besides, an increase in the proportion of carbon used for fatty acid production can also be done to increase soybean oil content [4].

The binding of fatty acids to the glycerol backbone can be increased by increasing the expression of the diacylglycerol acyltransferase (DGAT) enzyme coding gene. DGAT enzymes in plants and other organisms are divided into two types based on their structure, namely DGAT1 and DGAT2. This enzyme functions to catalyze the fusion of acetyl-CoA acid with diacylglycerol. The combination is the final stage of triglyceride formation. [14,15, 16]. Increasing the merging of the two compounds can increase the number of triglycerides in soybeans.
An increase in soybean oil in the seeds can be done by inserting genes from other organisms that can accumulate enough oil. One of the organisms that can accumulate oil is the fungus *Umelopsis ramanniana*. The insertion of the DGAT enzyme from *U. ramanniana* into the soybean plant genome can be done to increase the oil content in soybeans. The insertion of the DGAT gene from *U. ramanniana* in soybean plants could increase soybean oil content from 20% to 21.5%. This increase is stable in 3 growing seasons in two different geographical locations. The results of the study showed that the insertion of the DGAT2 gene from *U. ramanniana* into soybean plants had little or no effect on soy protein levels [17]. This shows that soybeans that have taken oil can be used for food or feed because the protein content is not much different from soybeans in general.

The next stage that can be carried out after assembling PRG soybeans is conducting safety testing and marketing authorization. Regulation of GMOs in Indonesia is regulated in Indonesian Government Regulation No. 21 of 2005. The Indonesian government divides biodiversity into three aspects, namely environment, food, and feed [18]. To be able to circulate in the Indonesian market, GM soybeans must obtain the three permits (Food, Feed, and Environmental safety). All three permits are needed because GM soybean will be used for feed and food as well, besides just for making biodiesel. This is done as an effort to realize the biorefinery approach. If harvested soybeans are only used as a source of biodiesel raw materials, and not used for food and feed, then the permits for food and feed safety are not necessary.

Before applying for a permit, GM soybean must be tested. GM testing must be done in laboratories, Limited Test Facilities, or Limited Field Tests [18]. If it has passed the three stages of the test, then GM soybean has the potential to obtain a distribution permit. The procedures for assessment, release, and distribution, and use of GMOs are regulated in Indonesian Government Regulation Chapter V No. 21/2005. The assessment is carried out based on a written request submitted by the applicant to the authorized Minister or the head of the authorized Non-Ministry Government Institution. Furthermore, the authorized Minister or the head of the Non-Ministry Government Institution is authorized to submit requests for GMO environmental safety recommendations to the Minister responsible for the environmental sector. After that, the Indonesian Biosafety Clearing House as an Indonesian biological safety commission instrument announces the receipt of requests, processes, and summaries of the results of assessments in a place accessible to the community to provide an opportunity for the community to submit their responses. Indonesian biological safety commission then submits environmental safety recommendations to the Minister, recommendations for food safety or food security to the authorized Minister or the authorized Non-Ministry Government Institution Head. The authorized Minister or authorized Non-Ministry Government Institution Head is obliged to base his decision on the biosafety recommendations given by the Minister or Chairman of the Indonesian biological safety commission [18].

2.2. Biorefinery of Biodiesel Production from Soybean

The use of the biorefinery approach can be used in the production of biodiesel from soybeans. The biorefinery concept used in the production of biodiesel from soybeans is similar to the refinery approach used in the petroleum industry but uses renewable raw materials [19]. The biorefinery system can be categorized as a system that is sustainable because it uses renewable biomass in this process [20]. The soybean processing industry has the potential to implement biorefinery systems. Compared to other conventional raw materials, soybean contains many components needed to make a biorefinery system, such as virgin oil extract from transesterification of soybean oil that can be used to produce biodiesel. Residues from soybean stalks can be used as raw material for biomaterial production, biofuel and other chemical compounds [21].

There are several processes involved in the soybean processing industry biorefinery. According to Cella et al. in 2002, the main stages in the soy biorefinery process are divided into four stages, which are degumming (hydration), neutralization (deacidification), bleaching, and deodorization [19]. Soybean processing with biorefinery systems can be a significant added value for the biodiesel industry from soybeans [19]. Soybean processing with a biorefinery system can produce residues in
the form of sap, soapstock, and spent bleaching earth (SBE) which can be processed into other goods. The concept of biorefinery in processing soybeans and the residues generated is shown in Figure 2, and the biorefinery concept in processing soybeans in more detail related to biodiesel can be seen in Figure 3.

Figure 2. Outline of biorefinery in soybean processing [19]
Figure 3. Biorefinery in soybean processing [19]

The first step in soybean processing is removing the skin and stem of the soybeans after harvest, and then soybean oil is extracted. Soybean oil extraction can be done using hexane compounds. The next step is chemical processing to remove unneeded oil components [19]. The initial stage of chemical processing of oil extracted (crude oil) is degumming. This process will produce residues in the form of soap stock. Soap stock consists of water, free fatty acids, neutral oils, phospholipids, non-saponified substances, proteins, and slimy substances [22]. Soap stock can be used to produce biosurfactants and rhamnolipids. Besides, soapstock can also be processed into biodiesel [19].

The oil that has undergone a degumming process is then put into the bleaching process to remove residues in the form of soap and phosphate, oxidize ketone body compounds, and break down peroxide into carbonyl compounds. The residue generated from this process is spent bleaching earth (SBE). SBE can be used as a material for making biodiesel. The oil that has passed the bleaching stage then enters the deodorizing stage. The purpose of deodorizing is to remove aldehydes, ketones, fatty acids, tocopherols, and sterols. This process residue is SODD (soybean oil deodorizer distillate). SODD contains many free fatty acids that have the potential as a source of biodiesel production, tocopherol which is widely used in the cosmetics industry, and sterols that are widely used in vitamin supplement formulas in the cosmetics industry. Also, glycerol compounds can be obtained from the byproducts of processing residue compounds into biodiesel [19].

The soybean oil processing industry can also utilize residues in the form of solid. Soybean stalks can be used as organic fertilizer for subsequent soybean planting, so it can help reduce production costs [19]. Soybean stalks can also be processed into sources of renewable raw materials, such as briquettes so that they can help reduce costs for energy needs. Residues in the form of soybean meal can also be processed into animal feed, food for human diets to industrial needs [23]. Soy protein extracted from solid residues during soybean oil extraction can also be used to produce glue, plastic to textile yarns [19]. Production of goods or materials from the soybean oil industry residues can help increase company revenue so that economic sustainability can be achieved. Besides, the use of residues can also reduce environmental pollution due to residual waste. Besides the use of GMO soybeans that have been modified in the DGAT gene or inserting the DGAT gene from other
organisms, has the potential to get a higher amount of oil. This can make the number of products produced more so that revenue for the company can increase.

2.3. Important Aspects of Biodiesel Production from GM Soybeans

Several things must be considered when producing biodiesel to replace diesel on the market. Things that must be considered include biodiesel production must have environmental benefits, economically competitive, have a high supply so that it can meet energy needs, and have a positive net energy balance ratio (NER) value [24,25]. Biodiesel production from GM soybeans with a biorefinery system can be beneficial to the environment. This is because most of the soybean oil production waste is reused as material for the formation of other products, so the amount of waste that released into the environment is less than the soybean oil industry in general. The NER value from soybean oil biodiesel production is also relatively high. The NER values of soybean oil production from various studies in the journal of Pradhan et al. in 2008 are presented in Table 1 [26].

| NER Value | Source                  |
|-----------|-------------------------|
| 2.51      | Ahmed et al. (1994)     |
| 3.21      | Sheehan et al. (1998)   |
| 0.79      | Pimentel & Patzek (2005)|
| 1.93      | Hill et al. (2006)      |

The NER value of biodiesel production from soybeans is relatively high. NER value is the value of how much energy is produced in each energy unit used. The biodiesel production industry has NER value of more than 1, so that means theoretically, biodiesel can be used to replace all energy used in producing it [25]. The NER value of biodiesel production from soybeans from various studies (Table 1) shows that many of the production has a NER value of more than 1. Based on data from Table 1, only Pimentel & Patzek research in 2005 has a NER value below one, which is 0.79 [27]. This can indicate that the production of biodiesel from soybeans has the potential to have a high NER value, making it feasible to produce.

The NER value can be used to calculate the value of the renewability of biodiesel production [26]. Based on the analysis of Pradhan et al., shown in Table 1, the production of biodiesel from soybeans can be said to be renewable and economically sustainable. The NER calculation of the GM soybean biodiesel production in Indonesia has not been performed, but if the GM soybeans used have more oil content, making biodiesel using soybean has the potential to increase the value of NER, because the oil products produced can be higher. With the application of the biorefinery system, the NER value can increase due to the reduction of waste released into the environment and the potential for new energy input for the industry.

3. Conclusions

The conclusion is that genetically modified soybeans can be used as a source of biodiesel. The use of the biorefinery approach can be carried out to increase the results obtained from the biodiesel industry, and become a sustainable industry, both economically and environmentally.

4. References
[1] PASPI Reascrh Team 2018 Monitor Isu Strategis Sawit 4 1361-1368
[2] Singh SP, Singh D 2010 Renewable and Sustainable Energy Reviews 14 200-216
[3] Adie MM, Krisnawati A 2014 International Journal of Renewable Energy Development 3 37-43
[4] Clemente TE, Cahoon EB 2009 Plant Physiology 151 1030-1040
[5] Jaworski J, Cahoon EB 2003 Curr Opin Plant Biol. 6 178–184
[6] Damude HG, Kinney AJ 2008 Physiol Plant. 132 1-10
[7] Bikker P, van Krimpen MM, van Wikselaar P, Houweling-Tan B, Scaccia N, van Hal JW, et al. 2016 J Appl Physcol. 28 3511–3525
[8] Balina K, Romagnoli F, Blumberga D 2017 Energi Procedia 128 Valttere S (editor) 504-511
[9] Haksawi PP, Susanto GWA, Taufiq A 2017 Penelitian Pertanian Tanaman Pangan 1 233-242
[10] Krisnawati A, Adie MM 2009 Iptek Tanaman Pangan 4 222-235
[11] Widyasakta AP 2018 SKRIPSI (Malang : Universitas Islam Negeri Maulana Malik Ibrahim)
[12] Suhartina 2005 Perkembangan dan Deskripsi Varietas Unggul Kedelai 1918–2004 Ade MM, Saleh N et al. (editor) (Malang : Balai Penelitian Kacang-kacangan dan Umbi-umbian. Malang).
[13] Heriyanto, Rozi F, Krisdiana R 2008 Prosiding Seminar Nasional Pengembangan Kacang-kacangan dan Umbi-umbian 61-75
[14] Cases S, Smith SJ, Zheng YW, Myers HM, Lear SR, Sande E, Novak S, Collins C, Welch CB, Lusis AJ, et al. 1998 Proceedings of the National Academy of Sciences of the United States of America 95 13018–13023
[15] Cases S, Stone SJ, Zhou P, Yen E, Tow B, Lardizabal KD, Voelker T, Farese RV Jr. 2001 Journal of Biological Chemistry 276 38870–38876
[16] Lardizabal KD, Mai JT, Wagner NW, Wyrick A, Voelker T, Hawkins DJ 2001 Journal of Biological Chemistry 276 38862–38869.
[17] Lardizabal K, Effertz R, Levering C, Mai J, Pedroso MC, Jury T, Aasen E, Grus K, Bennett K 2008 Plant Physiology 148 89–96
[18] Estiati A, Herman M 2015 Analisis Kebijakan Pertanian 13 129-146
[19] Pessoa FLP, Villardi H, Calixto EEdS, Vieira ED, de Souza ALB, Machado BAS 2019 IntechOpen
[20] Parada MP, Osseweijer P, Duque JAP 2017 Industrial Crops and Products 106 105-123
[21] Abdulkhani A, Alizadeh P, Hedjazi S, Hamzeh Y 2017 Renewable and Sustainable Energy Reviews 75 1269-1280
[22] Nitschke M, Costa SGVAO, Haddad R, Gonçalves LAG, Eberlin MN, Contiero J. 2005 Biotechnology Progress 21 1562-1566
[23] Liu K 1997 Soybeans Chemistry, Technology and Utilization (Dordrecht: Springer Science+Business Media Dordrecht) 556
[24] Hill J, Nelson E, Tilman D, Polasky S, Tiffany D 2006 Proc Natl Acad Sci. 103 11206–11210
[25] Schmer MR, Vogel KP; Mitchell RB, Perrin RK 2008 Proc Natl Acad Sci. 105 464-469
[26] Pradhan A, Shrestha DS, Van Gerpen J, Duffield J 2008 American Society of Agricultural and Biological Engineers 51 185-194
[27] Pimentel D, Patzek T 2005 Natural Resources Research 14 65-76