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Original Research Article

Bioconversion of Black Soldier Fly (BSF) from Organic Waste Composting into Biodiesel Assisted by Whole Cell Microbial Lipase Biocatalyst through Direct Transesterification Process

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Abstract

Black Soldier Fly or Hermetia Illucens (BSF) is known for its potential as a biological agent that helps in the process of natural conversion of organic waste. Resulting compost and a large number of BSF colonies, potentially to be use as a non-food raw material in bioenergy production. The Method is BSF conversion experiment by direct transesterification reaction using Lab-scale bioreactor in 100ml three bottle neck flasks, the solution mixture consists of BSF powder, immobilized whole cell biocatalyst, and solvent. The Result is although in appearance the structure of the test mixture solution did not show an oily character, but based on the viscosity and density test, the test solution had density value 0.81 g/cm^3 and viscosity value 1,024 mm^2/s which are above the value of the viscosity and density of methanol but still below the value of the viscosity and density of both SNI and EN14214 biodiesel standard, this could be due to insufficient separation or reaction, imperfect condition, and impurities that are still present in biodiesel products. It can be concluded that a transesterification reaction has occurred in this trial, however further analysis and more experiments are required to definitely conclude the changes in biodiesel production.

Keywords: Black soldier fly; biodiesel; organic waste; whole cell biocatalyst; direct transesterification

1. Introduction

Black Soldier Fly (BSF) is known for its potential as a biological agent that helps in the process of natural conversion of organic waste (Oduro-Kwarteng et al., 2018; Salmanl et al., 2020). In this process, Black Soldier Fly (BSF) is naturally attracted to the smell released by organic waste so that it perches and multiplies (colonizes). The bioconversion process occurs through metabolic reactions where Black Soldier Fly (BSF) insects utilize organic waste as a source of food nutrients in the process of growth and development, the larval stage, Black Soldier Fly (BSF) has the largest ability to eat nutrients in a relatively fast time, thus able to reduce organic waste volume (Yuwono and Mentari, 2018). The final result of conversion is compost and large number even thousands of Black Soldier Fly (BSF) colonies, this condition allows the use of Black Soldier Fly (BSF) as a non-food raw material in the manufacture of bioenergy. Black Soldier Fly (BSF) has bioenergy potential as a producer of biogas (Bulak et al., 2020) and biodiesel (Sawangkeaw and Ngamprasertsith, 2013). Some insects including the Black Soldier Fly (BSF) have been known to have a high fat content that can be converted into bioenergy (Franco et al., 2021;

Longyu Zheng, 2011; Manzano-Agugliaro et al., 2012; Wang et al., 2017). Three major fatty acid groups owned by Black Soldier Fly (BSF) are lauric fatty acids (C12: 0), palmitic fatty acids (C16: 0) and oleic fatty acids (C18: 0) (Nguyen et al., 2018; Sawangkeaw and Ngamprasertsith, 2013).

Based on the study analyzed the dynamic state of fat content and fatty-acid composition of BSF larvae in eight different stages, from genetic and molecular mechanism found that several putative genes are involved in the formation of pyruvate, acetyl-CoA biosynthesis, acetyl-CoA transcription, fatty-acid biosynthesis, and triacylglycerol biosynthesis. The four vital metabolic genes that are associated with fat accumulation were identified (Zhu et al., 2019). The late prepupa stage exhibited the highest crude fat, with lauric acid being the main component, thus promising to biodiesel conversion the best (Leong et al., 2018; Zhu et al., 2019). However only 70% of extractable oil (lipids) from BSF are able to convert to biodiesel through transesterification (Mohan et al., 2023), even though produced 80-94% yield which parameter met standard from some international standard for biodiesel (Li et al., 2011; Mohan et al., 2023; Nguyen et al., 2018; Park et al., 2022; Yusaf et al., 2022).

The conversion of Black Soldier Fly (BSF) fat into biogas has quite good potential, but the results of biogas vary according to the type of nutrients provided. Previous research found that the final result of biogas potential was 412.5 ± 5.1 ml g-1 vs and methane gas of 177.2 ± 18.3 ml g-1 (Bulak et al., 2020). However, Black Soldier Fly (BSF) conversion to biodiesel is more desirable considering that biodiesel is classified as clean energy, reduces pollutant emissions when mixed with diesel, effective operating costs, high conversion value, improves bioenergy characteristics, and can be used in various nutritional media (Ramli et al., 2017), and fatty acid conversion of Black Soldier Fly (BSF) to biodiesel has the density, viscosity, flash point and cetane index owned by international standard biodiesel, namely EN14214 (Li et al., 2011; Nguyen et al., 2018). Biodiesel conversion from Black Soldier Fly (BSF) usually throughout esterification-transesterification reaction (He et al., 2022; Ishak et al., 2018; Kamari et al., 2020; Li et al., 2011; Lim et al., 2022; Mohan et al., 2023; Park et al., 2022; Surendra et al., 2016), however direct transesterification recently used for biodiesel conversion of BSF due to simplicity stages in the process, cost efficiency, and Energy Conservation (Nguyen et al., 2018; Sitepu et al., 2023).

Based on chemical reaction process, the conversion commonly uses acid-base solvent or enzyme biocatalyst (Mangindaan et al., 2022). acid-base solvent commonly used are NaOH (Hong et al., 2018; Kamari et al., 2020; Li et al., 2011; Mohan et al., 2023; Wang et al., 2017) KOH (Mohan et al., 2023), potassium hydroxide (Sitepu et al., 2023), petroleum ether (Surendra et al., 2016), H_2SO_4 (Li et al., 2011; Mohan et al., 2023; Park et al., 2022), n-hexane:methanol (Nguyen et al., 2018), biochar acid catalyst (Leong et al., 2018), As for enzyme biocatalyst often used are pure enzyme synthesis or enzyme extracted from microbe. Some of it Novozym 435, an immobilized enzyme reacted to methanol produced 92.5% biodiesel which all the properties met the standard EN 14214 (Nguyen et al., 2017), Lipase SMG1 and Lipase Eversa Transform 2.0, free lipase reacted with methanol in 25°C achieved to 98.45% after 8h reaction (He et al., 2022).

Bioconversion of Black Soldier Fly into Biodiesel through Direct Transesterification are recently studied (Nguyen et al., 2018; Sitepu et al., 2023), However assisting process by whole cell biocatalyst haven't been studied in conversion of BSF into biodiesel. But recently assisting process by whole cell biocatalyst were studied and given as best as biodiesel yield resulted as from non or catalytic reaction (Almyasheva et al., 2018; Jia et al., 2018) therefore this study was conducted. Whole cell biocatalyst recently studied because of added value gain in the process by high selectivity, easy product separation, cost efficiency, lipase produce stability (Yan et al., 2012) catalytic efficiency, Repeated use (Ban et al., 2002), milder operational conditions, low impact on the environment (Anteneh and Franco, 2019), In addition to that, immobilization cell method help Enzyme or microbes stability (Madavi et al., 2021; Parwata and Oviantari, 2016; Sun et al., 2019) production and repeated use (He et al., 2016), reduce enzyme associated process costs or cost-effective (Fukuda et al., 2008; Jia et al., 2018) up-scale stability production (Srimhan and Hongpattarakere, 2023). There are a wide range of Microbial diversity approved to be whole cell biocatalyst agent in biodiesel conversion from bacteria (Parwata and Oviantari, 2016), fungi (Çağatay et al., n.d.; Yan et al., 2014), actinobacteria (Anteneh and Franco, 2019; Faturahman, 2019), diatom (Saranya and Ramachandra, 2020), etc. The outlook for researches Black Soldier Fly (BSF) Conversion into Biodiesel from 2011-2023 are served in **Table 1**.

Table 1. The outlook of BSF conversion into biodiesel

Article	Solvent	Catalyst	Reaction	Time	Temp	Yield	Met Standard	Reference
From organic waste to biodiesel: Black soldier fly, Hermetia illucens, makes it feasible	methanol	H_2SO_4 NaOH	Esterification - transesterification	1 h	85 °C	91.4 g	EN14214	(Li et al., 2011)
Lipase-catalyzed synthesis of biodiesel from black soldier fly (Hermetica illucens): Optimization by using response surface methodology	methanol	Novozym 435 (Immobilized Enzyme)	Transesterification- response surface methodology	9.48 h	26 °C	92.5 %.	EN14214	(Nguyen et al., 2017)
Insect biorefinery: a green approach for conversion of crop residues into biodiesel and protein	methanol	H_2SO_4 NaOH	Esterification - transesterification	30 min	65 °C	90%	EN 14214	(Wang et al., 2017)
New Approach to Improving Fuel and Combustion Characteristics of Black Soldier Fly Oil.	methanol	H_2SO_4 NaOH	Esterification - transesterification	1 h	75 °C	95 %	ASTM D6751	(Hong et al., 2018)
Optimisation of biodiesel production of Black Soldier Fly larvae rearing on restaurant kitchen waste	methanol	H_2SO_4 NaOH	Esterification -two-step transesterification using Response Surface Methodolog	68 min	60 °C	96%	NA	(Ishak et al., 2018)
Biodiesel Derive Bio-oil of Hermetia illucens Pre-pupae Catalysed by Sulphonated Biochar	methanol	Sulfonated Biochar n-hexane	Esterification - transesterification	2 h	500°C	95.6 %	NA	(Leong et al., 2018)
Direct transesterification of black soldier fly larvae (Hermetia illucens) for biodiesel production	n-hexane and methanol	NA	Direct transesterification	90 min	120 °C	94.14 %	EN 14214	(Nguyen et al., 2018)

Article	Solvent	Catalyst	Reaction	Time	Temp	Yield	Met Standard	Reference
Optimisation and characterisation studies of biodiesel production from black soldier fly larvae fed by soya residue.	methanol	Sulfuric and NaOH	Esterification -two-step transesterification response surface methodolog	32 min	60°C	96%	NA	(Kamari et al., 2020)
Transesterification synthesis of high-yield biodiesel from black soldier fly larvae by using the combination of Lipase Eversa Transform 2.0 and Lipase SMG1	methanol	Lipase SMG1 and Lipase Eversa Transform 2.0	Enzymatic transesterification	8 h	25 °C	98.45 %	EN 14214	(He et al., 2022)
Biodiesel Production From The Black Soldier Fly larvae grown on food waste and its fuel property characterization as a potential transportation	methanol	H_2SO_4	(Trans)esterification	6 h	70°C	86.51 %	Korea fuel standard (KS M 2965) except oxidation stability and EN 14214	(Park et al., 2022)
Black Soldier Fly Larvae (Hermetia illucens) for Biodiesel and/or Animal Feed as a Solution for Waste-Food-Energy	Methanol (most common)	H_2SO_4 NaOH Lipase (Enzyme Catalytic Process)	Esterification - transesterification (most common)	1 min-12 h	26-390°C	34-97%	NA	(Mangindaan et al., 2022)
Black soldier fly (Hermetia illucens) larvae as potential feedstock for the biodiesel production: Recent advances and challenges	Methanol (most common)	H_2SO_4 NaOH KOH Lipase or Protease (Enzyme Catalytic Process)	Esterification - transesterification (most common)	30 min-48 h	24-120°C	48-98%	EN 14214 KS M 2965 ASTM D6751	(Mohan et al., 2023)
Controlled crushing device-intensified direct biodiesel production of Black Soldier Fly larvae.	methanol	NaOH	Direct transesterification using a controllable crushing device (CCD)	20 min	room temperature 20–25 °C	93.8 %	EN 14214 & ASTM D6751	(Sitepu et al., 2023)

2. Methodology

BSF to be used is BSF obtained from the composting of organic waste that is turned off by watering it with hot water and soaking for 2 hours, and then dried in the sun for several days (2-3 days depend on the weather) until BSF is found in dry condition (crisp in the texture) and can be stored for later use. Total weight of BSF in dry condition are around 30% reduced from total fresh weight, from one batch of BSF harvested reach 2 kg of BSF fresh larvae and resulting around 650gr of dry BSF larvae. BSF when it will be used is crushed into powder. This powder will be used as a conversion raw material

Figure 1. BSF larvae (pupae) preparation for biodiesel production test

The microbial cultures used were *Aspergillus niger* and *Trichoderma Koningii* (T2). Previously isolated and identified from the screening process of rotting palm fruit, and lipase activity tests were carried out on the isolates obtained. So that the culture isolates of *Aspergillus niger* and *Trichoderma Koningii* (T2) were obtained which had the highest lipase activity.

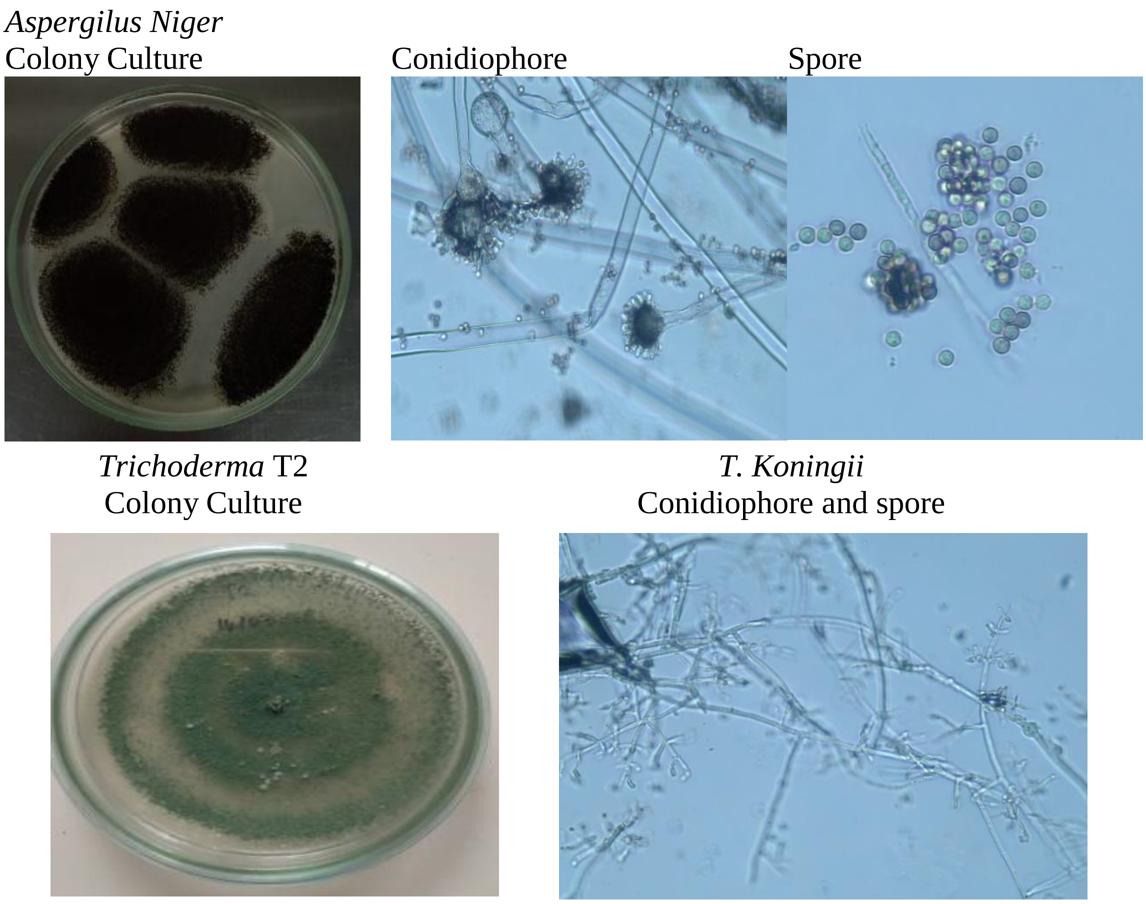

Figure 2. Microbe culture for biodiesel production test

Before the conversion test experiment, microbial lipase preparation was carried out by immobilizing microbial cells using Biomass Support Particles (BSPs). Microbial cell immobilization is carried out using BSPs 7 mm cubes of polyurethane foam with particle vacuums of more than 97% and pores of 100-500mm size. Immobilization of mycelium inside polyurethane foam is carried out by submerge cultivation in a growing medium of 13.2g sunflower oil, 7.4g soybean flour, 6.2g yeast extract in 1 L tap water. Microbial culture tests were carried out on rotary shakers at 220rpm and 30°C in 750mL Erlenmeyer tubes, containing 200mL of nutrient media and 1g BSPs for 72 hours. The immobile mycelium is then filtered, washed with 0.1 M phosphate buffer (pH 6.5) and freeze-dried (Almyasheva et al., 2018).

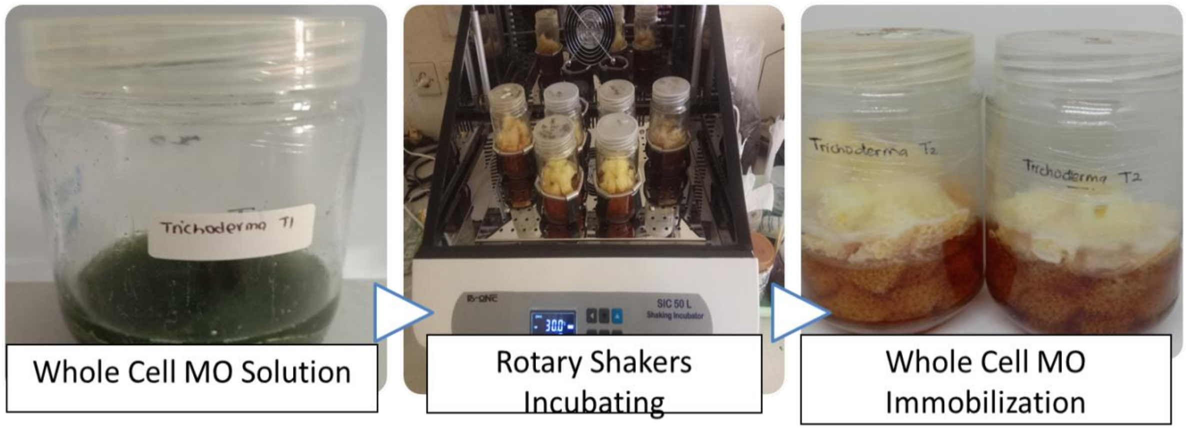

Figure 3. Whole cell immobilization procedures

Furthermore, the Black Soldier Fly (BSF) Biodiesel Conversion Test was carried out by conducting conversion experiments using Direct Transesterification reactions using small-scale bioreactors which referred to research conducted by (Xiao et al., 2011) with modifications. The solution mixture consists of BSF powder, biocatalyst, and solvent. The dose of BSF powder is 350g and solvent in the form of methanol 45 ml in 100ml three neck boiling flask. Then incubated at 40°C for 144 hours in waterbath. After the reaction, the determination of bioenergy yield from the reaction will be analyzed referring to the parameters of SNI 7182: 2015 concerning Biodiesel.

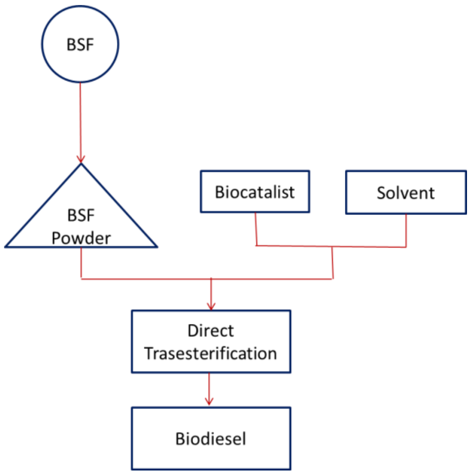

Figure 4. Outlook of bioconversion BSF into biodiesel research method

3. Result and Discussion

Bodiesel production testing was carried out by carrying out the direct transesterification experimental process, by reacting methanol solvent with the substrate, namely BSF powder, the work process was assisted by the addition of a lipase test microbial cell biocatalyst that had been immobilized. The experimental process was carried out by incubating the mixture at 40°C for 144 hours in a water bath.

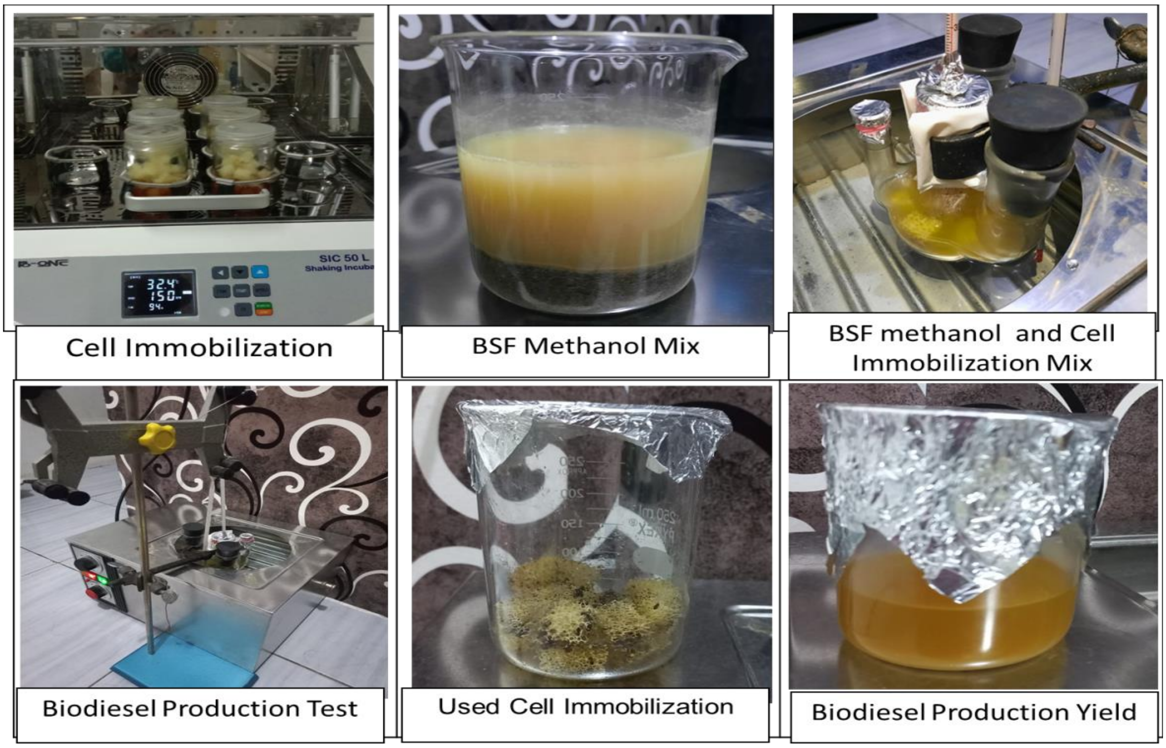

Figure 5. BSF biodiesel production test.

After the experiment, however, it was not possible to draw conclusions about the results obtained with certainty for the biodiesel test results obtained, because in appearance the biodiesel production test mixture liquid did not have an oily texture. Therefore, further analysis is needed such as FAME analysis with GC, analysis of Viscosity, density, Flash Point, Ester Content, Iodine Number, Free glycerol content, total glycerol content, and acid number. Based on the follow-up test which focused on the viscosity and density test first, it was found that the density value in the test mixture solution was 81 g/cm^3 and the viscosity value was 1,024 mm^2/s. : 2015 and the European Biodiesel standard EN14214, it can be concluded that the viscosity and density values of the test mixture solution did not meet the characteristic biodiesel standards. When compared with the viscosity and density values of methanol, namely with a density value of 0.790-0.800 g/cm^3 (Silaban and Makalalag, 2020), the density of the test mixture solution is above the standard methanol density, but still below the standard Biodiesel value. however, the test solution's viscosity value is above methanol's viscosity, where methanol's viscosity value is 0.55 mm^2/s (Silaban and Makalalag, 2020).

One of the possibility is, this can be caused by compounds such as soap, catalysts, and methanol which are thought to still be in the biodiesel produced due to incomplete separation or reactions and there are still impurities present in the biodiesel product (Silaban and Makalalag, 2020). However, to state that there is or is not a change from the fatty acids owned by BSF to biodiesel, further analysis of the chemical structural components is required in the test mixture solution such as GC analysis, FTIR, and so on so that it cannot be concluded with certainty that the trial process for biodiesel production from BSF fatty acids with the help of microbial lipase is successful or not. So an analysis of the possibilities that have the potential to become problems that occur in this biodiesel conversion trial is carried out so that the conversion does not occur in full.

Table 2. Parameter of biodiesel production based on SNI 7182:2015

Parameter	Unit	Value
		SNI of Biodiesel
Density (40°C)	g/cm³	0.850-0.890
Viscosity (40°C)	mm²/s	2.3-6.0
Flash Point	°C	min 100
Free Glycerol Content	%-mass	max 0.02
Total Glycerol Content	%-mass	max 0.24
Acid Value	mg	max 0.8
Fatty acid ethyl ester content	KOH/g	Min 96.5
Iodine Value	gI²/100g	max 115

Source: (Badan Standardisasi Nasional, 2015)

Table 3. Parameter of biodiesel production test

Parameter	Unit	Value			
		Result test	SNI of Biodiesel	EN14214	Methanol
Density (40°C)	g/cm³	0.81	0.850-0.890	872 – 878	0.790-0,800
Viscosity (40°C)	mm²/s	1,024	2.3-6.0	3.5-5.0	0.55

Source : (Badan Standardisasi Nasional, 2015; *FAME EN 14214:2012*, 2012)

Based on the analysis of the use of the method in this biodiesel production test experiment from BSF, the direct transesterification method is used because based on the latest developments in biodiesel conversion research using the direct transesterification method where the process steps are fewer by only going through one process step, using methanol which functions as a solvent and transesterification reactant. and with the help of various catalysts including biocatalysts (Katre et al., 2018; Nguyen et al., 2018; Rizwanul Fattah et al., 2020; Sun et al., 2017; Zhang et al., 2015). Generally, the biodiesel process from BSF involves an esterification-transesterification process (a two-step process) that converts free fatty acids into biodiesel (methyl ester), with the help of acid and base catalysts. However, this process requires oil extraction and refinement which is costly and produces high yields. low (Nguyen et al., 2018; Zheng et al., 2012). Thus the direct transesterification method can be applied and has been successfully carried out in other previous studies.

In the transesterification reaction, the influencing factors include the characteristics of the substrate, namely water content, and composition of fatty acids, type, and concentration of catalyst, the molar ratio of oil and alcohol, solvent, co-solvent, temperature, reaction time, and mixing composition (Ondul et al., 2015; Rizwanul Fattah et al., 2020). In this study, the direct transesterification method was used based on research used by (Xiao et al., 2011). In this study, no co-solvent was used because the basic assumption in this study was optimizing the utilization of biocatalyst activity from microbes, where biocatalysts are biological substances that function to accelerate the rate of chemical reactions without affecting these living organisms (Faturahman, 2019; Ondul et al., 2015; Rizwanul Fattah et al., 2020). Biocatalysts have relatively good pH and temperature tolerances and have high selectivity for substrate and product stereochemistry (Jemli et al., 2016).

The biocatalyst used in the process of converting fatty acids into biodiesel is a biocatalyst that has lipase activity. Lipase is an enzyme that has high activity in various chemical reactions, including hydrolysis, esterification, and transesterification (Wahyuningsih et al., 2015). There are two lipase enzymatic reactions used in the synthesis of biodiesel, namely, extracellular and intracellular. Extracellular lipase is an enzyme extracted and purified from microorganisms. Meanwhile, intracellular lipase is an enzyme that remains inside (the cell wall or inside the cell). Extracellular lipase requires high costs and complex separation and purification techniques (Rizwanul Fattah et al., 2020). Meanwhile, intracellular lipase tends to be preferred and is commonly used because the lipase enzyme is still in the microbial cell and will continue to produce as long as the microbe is alive, so it is suitable for processes that are carried out continuously, thus production is economically efficient and environmentally friendly. So that the biocatalyst technique using the whole cell biocatalyst technique used in this study is appropriate to apply, and this has been supported by research data on biodiesel conversion using whole-cell biocatalysts which have succeeded in producing biodiesel with high yields (Almyasheva et al., 2018; Xiao et al., 2011).

Various types of microorganisms are used to produce lipase as a biocatalyst including *Rhizomucor miehei* (De Vasconcellos et al., 2015; Tacias-Pascacio et al., 2017). *Pseudomonas cepacia* (Kumar et al., 2020), *P. fluorescens* (Ferrero et al., 2020), *Thermomyces lanuginosus* (Gumba et al., 2016), *Candida rugose* (Sánchez-Bayo et al., 2019), C. Antarctica (Sánchez-Bayo et al., 2019), *Rhizopus sp.* (Wahyuningsih et al., 2015), *Aspergillus niger* (Almyasheva et al., 2018; Regner et al., 2019), *Trichoderma sp.* especially *T. reesei, Harzianum, Viride* (Riwayati et al., 2012), actinobacteria or actinomycetes *Streptomyces exfoliates* and *Nocardiopsis alba* (Faturahman, 2019), Yeast (Deeba et al., 2020), *Cladosporium tenuissimum* (Saranya and Ramachandra, 2020). This is in accordance with the results of screening and isolation of lipase-producing microbes obtained and used in the tests in this study, namely *Aspergillus niger* and *Trichoderma Koningii*.

Enzyme immobilization is a commonly used technology to contain and stabilize enzymes from harsh environmental conditions such as pH, temperature, chemical compounds, and agitation. There are three enzyme immobilization strategies that can be carried out, namely direct immobilization of surface modifications (Kumar et al., 2020), immobilization with beads (Ferrero et al., 2020), and enzyme immobilization on monoliths (Gumba et al., 2016). Immobilization of lipase enzymes can be carried out by physical absorption (Ondul et al., 2015; Tacias-Pascacio et al., 2017), covalent binding (Gumba et al., 2016), cross-linking enzyme aggregate (CLEA) (Gumba et al., 2016; Sánchez-Bayo et al., 2019), ion exchange (De Vasconcellos et al., 2015; Ondul et al., 2015), and entrapment methods (Gumba et al., 2016; Kumar et al., 2020; Ondul et al., 2015). This was also done in this research experiment by adopting the cell immobilization technique with the entrapment method using a supporting matrix media such as polyurethane foam (Xiao et al., 2011).

Another possible caused is temperature of the reaction on enzyme catalytic activity, likely it's because of the temperature help produce higher reaction rate and yield at a higher temperature, meanwhile higher temperature increased the more possible leads to enzyme inactivation (He et al., 2022). Based on several researches of BSF Conversion into Biodiesel on the **Table 1**, the temperatures for enzymatic catalytic reaction are 20-25°C for 8 hours (He et al., 2022). However, some other research on biocatalyst used on biodiesel production are able to used higher temperature and still obtained high yield of biodiesel. Such as 35-60°C for more than 24 h (Gumba et al., 2016), 40°C for 3-24 and 72 hours (De Vasconcellos et al., 2015; Xiao et al., 2011), 37°C for 6-20 hours (Ferrero et al., 2020), 30-37°C for 24-165 hours (Fukuda et al., 2008) and 30°C for 48 and 72 hours (Almyasheva et al., 2018; Riwayati et al., 2012). Therefore, the reaction temperature was set at 40°C in this study referred to *Aspergillus niger* whole cell biocatalyst assisted in biodiesel Production by (Xiao et al., 2011) is acceptable.

4. Conclusions

Although in appearance the structure of the test mixture solution did not show an oily character, based on the results of the viscosity and density test, the test solution had a value above the value of the viscosity and density of methanol but still below the value of the viscosity and density of biodiesel, this could be due to insufficient separation or reaction. Imperfect and there are still impurities that are still present in biodiesel products. It can be concluded that a Transesterification reaction has occurred in this trial, but to definitively conclude changes in biodiesel require further analysis.

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