GREEN-CO :BIODIESEL PRODUCTION FROM CHLORELLA VULGARIS AND N-HEXANE USING ULTRASONIC METHOD

Nayla Fathma Lathifa Azdyaputri
XII-8 SMA Negeri 3 semarang
nayla.azdyaputri@gmail.com

Safira Aulia Labibah
XII-10 SMA Negeri 3 semarang
safira.aulia@gmail.com

Guru Pembimbing 1: Yusuf Rahmad, S.Pd.

Abstract

Biodiesel, as a renewable fuels is one of the most important discovery to reduce the amount of greenhouse gas emissions and microalgae is an organism who plays a big role in the process of making biodiesel. Microalgae Chlorella vulgaris has high oil content amount for about 57%. Therefore, Chlorella vulgaris is a very potential material for biodiesel. This research was conducted by 6 processes: Lipid extraction (added N-Hexane solvent), Ultrasonication, Viscosity test, Density test, Acid number test, and Saponification number test. The lipid extraction process was conducted 2 times. The ultrasonication process resulting a nanoparticle size (smaller size) of extraction which are more effective to the biodiesel quality. The ratio of Chlorella vulgaris and N-Hexane are 1:4 and 1:8. This research resulted the ratio of 1:8 is more effective than the ratio of 1:4 to become the raw material for biodiesel production.

Keywords*: Biodiesel, Microalgae, Chlorella vulgaris, Ultrasonication*

INTRODUCTION

The human populations would be increase every year. Meanwhile, humans are social creatures who need to mobilize. To fulfill their need of mobility, humans need transportation. Currently, transportation fuels are dominated by non-renewable fuels such as petroleum. In recent years, due to various environmental issues caused by fossil fuels and worldwide quest to increase use of renewable biofuels, microalgae have attracted tremendous attention in biotechnology research with regard to biofuel production, such as methane, biodiesel and biohydrogen (Chisti, 2007). Intensive consumption of non-renewable fuels can lead to scarcity someday. If no new reserves are found, oil supplies in Indonesia are expected to run out in the next nine years. Greenhouse gas emissions will increase, global warming will become more prevalent, climate change will occur, the environment will be massively polluted, and public health will be affected.

Biodiesel, an alternative fuel that is low in producing CO, CO2, NO, SO, and hydrocarbon emissions, can be a solution for this problems. Biodiesel is a renewable fuel oil, a renewable mono-alkyl ester derived from natural materials, such as plant oils and animal fats, through the process of transesterification process (Abbaszaadeh et al., 2012;Demirbas, 2010). Biodiesel is produced from vegetable oils that can be taken from plants or microalgae.
Microalgae, small photosynthetic organisms that can grow rapidly in water or other media. Microalgae are a very promising feedstock for biodiesel

production. Microalgae are single-celled photosynthetic organisms, it use light energy and carbon dioxide, with higher photosynthetic capacity more efficient than biomass production. Microalgae are microscopic organism with diameters between 3-30 μm. Microalgae is one of the vegetable oil-producing materials that is widespread in Indonesia. Microalga has various advantages over conventional oil crops plants. The oil content also reaches 70% of dry weight and does not require fertile soil to grow, making microalgae one of the materials that can be utilized as a raw material for biodiesel. The oil productivity of microalgae exceeds that of the best oil-producing plants (Converti et al., 2009).

Microalgal-based biodiesel is a potential renewable resource for displacement liquid transport fuels derived from petroleum (Christi, 2008). Microalgae is an autotrophic organisms that are able to photosynthesize such as high level plants, so they can also automatically reduce the amount of greenhouse gas emissions because the CO2 will be used in the photosynthesis process. From the photosynthesis process, solar energy can be converted into chemical energy, one of which is stored in the form of lipids.

There's an interesting species of microalgae to investigate, which is *Chlorella vulgaris*.*Chlorella vulgaris* has high oil content amount for about 57%. Therefore, *Chlorella vulgaris* is very potential material for biodiesel.

Table 1. Chemical content of various microalgae species in dry biomass (%)

Microalgae species	**Lipid content (%biomass)**
Chlorella emersonii	25-63
Chlamydomonas minotissima	57
Chlorella sp.	10-48
Chlorella vulgaris	5-58
Dunaleilla salina	6-25
Dunaleillaprimolicta	23
Dunaliella sp.	17-67
Nanochloris sp.	20-56
Nanochloropsis sp.	12-53
Schizochytrum sp.	50-77
Skelotonemacostatum	13-51
Pavtova salina	30
Pyrrosialeavis	69
Zitzschia sp.	45-47

Source: Kawaroe et.al, *MikroalgaPotensi dan Pemanfaatannya untuk Produksi Bio Bahan Bakar*, (Bogor :IPB Press, 2010), hlm. 15.

Recently, integrated approaches that overcome the technological-economic limitations of the current microalgal biodiesel production process, such as simultaneous harvesting/cell disruption using nanoparticles (Lee et al., 2014b). Nannoparticle can be used as disruption agents and catalysts in the production of biodiesel from microalgae. Disruption agent means as a disruptor of the microalgae cell wall so that the lipids contained in it can be extracted more easily. Catalyst means to accelerate the reaction that converts lipids into biodiesel. Thus, the use of nanoparticles can increase the efficiency of lipid extraction from microalgae and its conversion into biodiesel. Some recent studies also suggest that engineered nanoparticles can transport DNA and chemicals into algae cells and it has been found that the size of nanoparticles is inversely proportional to the number of molecules present. Nanoparticles have a size range below 100 nm so that they can increase the growth and lipid production of microalgae. This paper will discuss the effectiveness comparison of Chlorella

vulgaris microalgae mixed with n-hexane by ratio of 1:2, 1:4, and 1:8 as feedstock for nanoparticle-based biodiesel.

Research Purpose

1. Knowing the number of viscosity and density from the Microalgae Chlorella vulgaris with N-Hexane Solvent in the ratio of 1:4 and 1:8 compared with SNI Rate.
2. Knowing the acid number and saponification number from the Microalgae Chlorella vulgaris with N-Hexane Solvent in the ratio of 1:4 and 1:8 compared with SNI Rate.
3. Analyze the the effect of amount from N-Hexane mixed with microalgae Chlorella vulgaris as solvent.
4. Analyze the most effective ratio of microalgae Chlorella vulgaris mixed with N-Hexane solvent in making biodiesel.

METHOD AND EXPERIMENTAL DETAILS

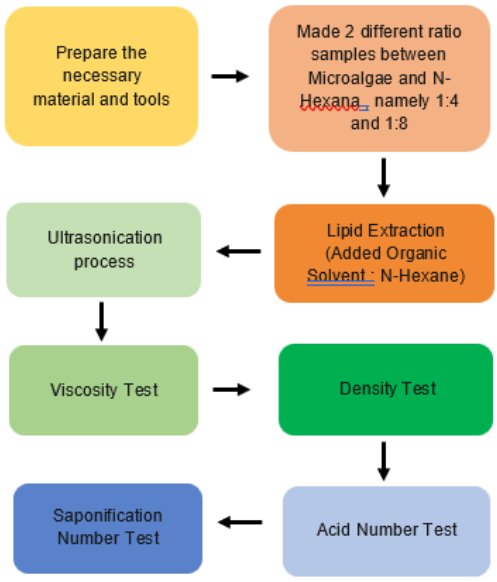
Time and Place

This research was conducted on July 2023 until November 2023. Held at Researcher's house and STIFAR (Sekolah Tinggi Ilmu Farmasi) Semarang.

Research Type

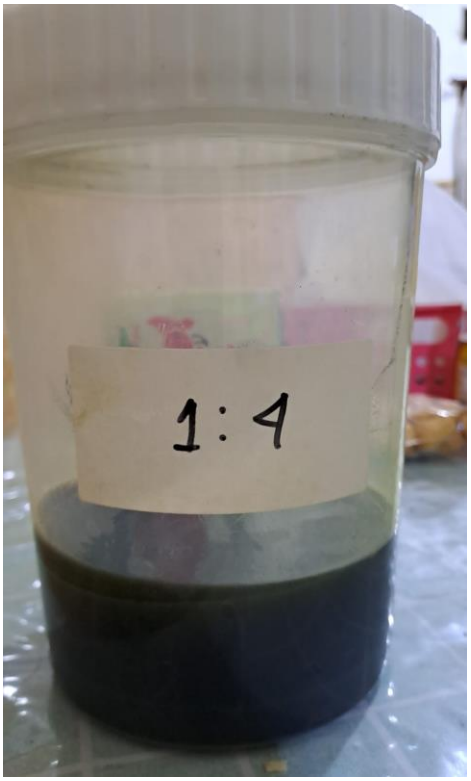
The type of our research are quantitative and qualitative. While, our research methodology is Experimental which include ultrasonication process, viscosity, density, acid number, and saponification number test.

Research Design



The chemical N-Hexane has analytical quality/specification (PA). N-hexane has been proven to be more optimal as a solvent extraction than Methanol. Resulting in a yield of 30.14 gr/ml while Methanol only produce 29.02 gr/ml. Therefore, N-hexane solvent can extract algae oil better than methanol solvent. This is because the nature of oil where it will be more soluble to non-polar solvent components, where n-hexane is more non-polar than methanol. The solubility is due to the Van Der Wall attractive force between the solvent and the solute, as with other alkane compounds, n-hexane is insoluble in water.

The experimental procedure was as follows: Preparing *Chlorella Vulgaris* powder as much as 80 g. The ultrasonic extraction device was assembled and placed with the algae mixed with the extraction solvent in a round bottom flask at 50 degrees Celcius and with an operating time of 1 hour.



Picture 1. The sample documentation after being ultrasonicated
Source : Researcher's documentation

In the ratio of 1:4, N-Hexane that was used is 320 mL. While In the ratio of 1:8, N-Hexane that was used is 640 mL. Then, it followed with the tests of viscosity, density, acid number, and saponification number.

Viscosity is the resistance of a fluid to changes in shape or movement of adjacent parts relative to one another. To summarize, viscosity is a measure of a fluid's resistance to flow. The acceptable viscosity range for biodiesel according to SNI is between 2,0 – 4.5 mm2/s (cp). To define the viscosity, an Ostwald Viscometer was used to determined the concentration of microalgae.

Picture 2. Viscometer Ostwald
Source : Researcher's documentation

b. Viscosity Measurement for the ratio of 1:4

Table 2. Viscosity Measurement Data for the Ratio 1:4

No	Sample	I	II	III	∑ Time
1.	Aquadest	63 second	64 second	63 second	63,33 second
2.	Sample X	72 second	72 second	74 second	72,66 second

Water density = 0.998 g/mL
Sample X's density = 0.6590 g/mL
η water = 0.89 cp (Handbook Of Pharmaceuticals Exipients, 766)

$$\eta \text{ Sample X} = \frac{t.Sample\ x\ \rho\ Sample}{t.water\ x\ \rho\ water} x\ \eta water$$

$$= \frac{72.66\ second\ x\ 0.6590\ g/mL}{63.33\ second\ x\ 0.998\ g/mL} x\ 0.89\ cp$$

$$= 0.7576\ cp$$

c. Viscosity Measurement for the ratio of 1:8

Table 3. Viscosity Measurement Data for the Ratio 1:4

No	Sample	I	II	III	∑ Time
1.	Aquadest	63 second	62 second	63 second	62.67 second
2.	Sample X	47 second	48 second	47 second	47.33 second

Water density = 0.998 g/mL
Sample X's density = 0.6674 g/mL

η water = 0.89 cp (Handbook Of Pharmaceuticals Exipients, 766)

η SampleX $= \frac{t.Sample\ x\ \rho\ Sample}{t.water\ x\ \rho\ water} x$ η water

$= \frac{47.33\ second\ x\ 0.6674\ g/mL}{62.67\ second\ x\ 0.998\ g/mL} x$ 0.89 cp

= 0.4494 cp

Density is a measure of the density or compressibility of a substance, which is a comparison between the mass and volume of the substance itself (Rahim and Indah, 2017). Based on SNI 7182:2015 Biodiesel, the density value must be below 0.850-0.890 g/mL. In this research, we measure the density number by using Pycnometer

Picture 2. Pycnometer
Source : Researcher's Documentation

a. Density Measurement for the ratio of 1:4

Weightof empty Pycnometer = 10.5269 *g*
Weight of Pycnometer + Aquadest = 21.0726*g*
Weight of Pycnometer + Sampel X = 17.4908*g*
Waterdensity at 20 degree Celcius = 0.998 g/mL (Indonesian Pharmacopoeia III edition, 96)

- **Determine the volume density of a pycnometer**

Weight of Pycnometer + Aquadest = 21.0726*g*
Weight of empty Pycnometer = 10.5269*g*
Weight ofAquadest = 10.5457*g*

Vol. Pycnometer = Vol. Water = $\frac{Weight\ of\ water}{\rho\ water}$ = $\frac{10,5457\ g}{0,998\ g/mL}$ = 10.5668*mL*

- **Determine the Density and Specific Weight of Sample X**

Weight of Pycnometer + Sample = 17.4908 g
Weight of empty Pycnometer = 10.5269 g
Weight ofAquadest = 6.9639 g

ρ Sample = $\frac{Weight\ of\ Sample}{Vol.Pycnometer}$ = $\frac{6.9639\ g}{10.1122\ mL}$ = 0.6590 g/mL

Sample specific gravity = $\frac{\rho\ Sampel}{\rho\ Air}$ = $\frac{0.6590\ g/mL}{0.998\ g/mL}$ = 0.660

b. Density Measurement for the ratio of 1:8

Weightof empty Pycnometer = 11,6444 *g*
Weight of Pycnometer + Aquadest = 21,7364 *g*
Weight of Pycnometer + Sampel X = 18,3933 *g*
Waterdensity at 20 degree Celcius = 0.998 g/mL (Indonesian Pharmacopoeia III edition, 96)

- **Determine the volume density of a pycnometer**

Weight of Pycnometer + Aquadest = 21,7364 *g*
Weight of empty Pycnometer = 11,6444 *g*

Weight ofAquadest = 10,0920 *g*

Vol. Pycnometer = Vol. Water = $\frac{Weight\ of\ water}{\rho\ water}$ = $\frac{10,0920\ g}{0,998\ g/mL}$ = 10,1122 *mL*

- **Determine the Density and Specific Weight of Sample X**

Weight of Pycnometer + Sample = 18,3933 g
Weight of empty Pycnometer = 11,6444 g
Weight ofAquadest = 6,7489 g

ρ Sample = $\frac{Weight\ of\ Sample}{Vol.Pycnometer}$ = $\frac{6,7489\ g}{10,1122\ mL}$ = 0,6674 g/mL

Sample specific gravity = $\frac{\rho\ Sampel}{\rho\ Air}$ = $\frac{0.6674\ g/mL}{0.998\ g/mL}$ = 0,669

Acid number is a number that indicates the amount of free fatty acids contained in a fat or oil, which is usually associated with the hydrolysis process of the fat or oil (Fitri and Fitriana, 2019). As stated in SNI, the value of acid number should not exceed 0,5 mg-KOH/g. The total acid number is determined by titrating the sample dissolved in an organic solvent (ethanol: Chloroform 1:1) Using KOH 0.1 N, PP indicator. Acid number measurement in this study was determined by a several process. First process is Determine the Level of Acid Number. This process begin by weighed carefully 10 grams of sample put in erlenmeyer, add 20 ml of neutral ethanol, added 3 drops of 1% PP indicator, and titrate it with 0.1N KOH solution. The second process is the process of Standarization KOH by Oxalic Acid. This process begin by pipetting 10.0ml of 0.0999N Oxalic Acid solution into Erlenmeyer, add 40ml of CO2-free distilled water, add 1% PP indicator, then titrate it with 0.1N KOH solution.

a. Acid Number Measurement for the ratio of 1:4

- KOH Standarization with Oxalic Acid

Table 4. KOH Standarization with Oxalic Acid Data for the Acid Number Measurement (Ratio 1:4)

NO	Oxalic Acid Volume	Titrant volume readings	Titrant volume
1	10.0	0,00 – 10.50	10.50
2	10.0	0,00 – 10.45	10.45
3	10.0	0,00 – 10.50	10.50

Average titrant volume : 10.48 mL

- Sample rate determination with KOH 0,1 N

Table 5. Sample rate determination with KOH 0.1 N for the Acid Number Measurement (Ratio 1:4)

NO	Weight of weighted bottle + substances	Weight of weighting bottle + remnant	Weight of substance	Titrant volume reading	Titrant volume	Rate %
1	42.1225	32.1211	10,0014	0,000- 2.600	2.600	0.14
2	41.2474	31.2254	10,0220	0,000- 2.600	2.600	0.14
3	43.4929	33.1653	10,3276	0,000- 2.600	2.600	0.13

- Calculation

1. Standarization

V1 x C1 raw primary = V2 x C2 raw secondary
10.0ml x 0.1000N = 10.48mLx C2
C2 = 0.0954 N

2. Rate Determination

SAMPEL 1 = $\frac{(V\ xN)KOH \times BM\ KOH \times 100\%}{Mg\ Sampel}$

$= \frac{(2.600 \times 0.094) \times 56{,}1 \times 100\%}{10001.4}$

= 0.14%
Average rate = (0.14+0.14+0.13)/3
= 0.14%
Therefore, the sample contains Acid Number 0.14%
(SNI Requirements cannot be more than 5.0 %)

b. Acid Number Measurement for the ratio of 1:8

- KOH Standarization with Oxalic Acid

Table 6. KOH Standarization with Oxalic Acid (Ratio 1:8)

NO	Oxalic Acid Volume	Titrant volume readings	Titrant volume
1	10.0	0.00 – 11.70	11.70
2	10.0	0.00 – 11.80	11.80
3	10.0	0.00 – 11.70	11.70

Average titrant volume : 11.75 mL

- Sample rate determination with KOH 0.1 N

Table 7. Sample rate determination with KOH 0.1 N for the Acid Number Measurement (Ratio 1:8)

NO	Weight of weighted bottle + substances	Weight of weighting bottle + remnant	Weight of substance	Titrant volume reading	Titrant volume	Rate %
1	44.3250	34.1516	10.1734	0.000- 6.000	6.000	0.28
2	43.8729	33.7723	10.1006	0.000- 6.700	6.700	0.32
3	34.4580	24.2881	10.1699	0.000- 6.400	6.400	0.30

- Calculation

1. Standarization Data

V1 x C1 raw primary = V2 x C2 raw secondary
10.0mLx 0.0999N = 11.75ml x C2
C2 = 0.0850 N

2. Rate Determination Data

SAMPEL 1= $\frac{(V\ x\ N)KOH \times BM\ KOH \times 100\%}{Mg\ Sampel}$

$= \frac{(6.000 \times 0.0850) \times 56.1 \times 100\%}{10173.4}$

= 0.28%
Average rate = (0.28+0.32+0.30)/3
= 0.30%
Therefore, the sample contains Acid Number 0.30%
(SNI Requirements cannot be more than 5.0 %)

Saponification number is the number of milligrams of KOH needed to saponify 1 gram of biodiesel sample (Dogra et al. 2005). Saponification rate measurement in this study was determined by a several method. The first method is the method of determine the rate. Begin by : Weighed carefully 10 grams of sample put in Erlenmeyer, add 20.0 ml of 0.5N KOH, then reflux it for 15 minutes then cooled, add 2 drops of 1% PP indicator, and titrate it with 0.5N HCl until the exact pink colour disappeared. Blank titration was performed. The second method is Standarization Method. It begin by carefully pipetted 10.0 ml of Na2B4O7 solution was put in Erlenmeyer, add 40 ml of CO2-free distilled water, add 2 drops of MR1% inductors, titrate it with 0.5N HCl solution until orange colour. And the last method is Blanko Titration

Method. It begin by pipetted 20.0 ml of 0.5N KOH into Erlenmeyer, reflux it for 15 minutes then cooled, add 2 drops of 1% PP indicator, then titrate it with 0.5N HCl solution until the exact pink colour disappeared

a. Saponification Number Measurement for the ratio of 1:4

1. Standarization Data

Table 8. Sample rate determination for the Saponification Number Measurement (Ratio 1:4)

NO	Na2B4O7 Volume	Titrant volume reading	Titrant Volume
1	10.0 mL	0.00 – 10.10	10.10
2	10.0 mL	0.00 – 10.10	10.10
3	10.0 mL	0.00 – 10.10	10.10

Titrant Volume Average = 10.10 ml

2. Rate Determination Data

Table 9. Sample rate determination data for the Saponification Number Measurement (Ratio 1:4)

NO	Weighed Bottle Weight & Substances	Weighed Bottle Weight & Residual	Weight of Substances	Titrant Volume Reading	Titrant Volume	Rate
1	38.7314	28.7124	10.0190	0,00 – 16.90	16,00	16.90
2	35.1922	25.1098	10.0824	0,00 – 17.30	15,90	17.30
3	37.4301	27.1123	10.3178	0,00 – 17.40	15,90	17.40

Calculations

1. Standarization Data

V1 x N1 raw primary = V2 x N2 raw secondary

10.0 x 0.5000 = 10.10 x N2

N2 = 0.5000 N

The real normality of HCL is 0.4964 N

2. Rate Determination Data

Saponification Number $= \frac{Blanko\ Vol - Sample\ Vol\ x\ N\ HCL\ x\ 56{,}1}{Gram\ Sample}$

$$= \frac{(16.10 - 16.00)\ x\ 0.5000\ x\ 56{,}1}{56.1}$$

$$= 1.33$$

So, the sample's saponification number is = 2.21

b. Saponification Number Measurement for the ratio of 1:8

2. Standarization Data

Table 10. Sample rate determination for the Saponification Number Measurement (Ratio 1:8)

NO	Na2B4O7 Volume	Titrant volume reading	Titrant Volume
1	10.0 ml	0.00 – 10.10	10.10
2	10.0 ml	0.00 – 10.10	10.10
3	10.0 ml	0.00 – 10.10	1010

Titrant Volume Average = 10.10 mL

3. Rate Determination Data

Table 11. Sample rate determination data for the Saponification Number Measurement (Ratio 1:8)

NO	Weighed Bottle Weight & Substances	Weighed Bottle Weight & Residual	Weight of Substances	Titrant Volume Reading	Titrant Volume	Rate
1	27.3159	25.2154	2.1005	0.00 – 16.00	16.00	1.33
2	21.8564	19.7701	2.0863	0.00 – 15.90	15.90	2.67
3	22.5256	20.4074	2.1182	0.00 – 15.90	15.90	2.63

Calculations

3. Standarization Data

V1 x N1 raw primary = V2 x N2 raw secondary

10.0 x 0.5014 = 10.10 x N2

N2 = 0.4964 N

The real normality of HCL is 0.4964 N

4. Rate Determination Data

Saponification Number $= \frac{Blanko\ Vol - Sample\ Vol\ x\ N\ HCL\ x\ 56{,}1}{Gram\ Sample}$

$$= \frac{(16.10 - 16.00)\ x\ 0.4964\ x\ 56.1}{2.1005}$$

$$= 1.33$$

So, the sample's saponification number is = 2.21

RESULT AND DISCUSSION

Table 12. Result All Test compared with SNI rate

No	Test type	Ratio		SNI
		1:4	1:8	
1	Viscosity Test	0.7576 cp	0.4494 cp	2.0-4.5 mm^2/s
2	Density Test	0.660 g/mL	0.669 g/mL	0.815-0.88 g/ml
3	Acid Number Test	0.14%	0.30%	< 5.0 %
4	Saponification Number Test	2.21	2.21	< 500 mg KOH /g

The data that was obtained are :

1. In the experiment of 1:4 (Chlorella vulgaris : N-Hexane solvent) shows =
   - Viscosity number of 0.7576 cp. It doesn't fulfill the SNI rate (2.0-4.5 mm^2/s).
   - Density number of 0.660 g/mL. It doesn't fulfill the SNI rate (0.815-0.88 g/ml).
   - Acid number rate of 0.14%. It fulfills the SNI rate (<5.0 %).
   - Saponification number of 2.21. It fulfills the SNI rate (<500 mg KOH/g).
2. In the experiment of 1:8 (Chlorella vulgaris : N-Hexane solvent) shows =
   - Viscosity number of 0.4494 cp. It doesn't fulfill the SNI rate (2.0-4.5 mm^2/s).
   - Density number of 0.669 g/mL. It doesn't fulfill the SNI rate (0.815-0.88 g/ml).
   - Acid number rate of 0.30%. It fulfills the SNI rate (<5.0 %).
   - Saponification number of 2.21. It fulfills the SNI rate (<500 mg KOH/g).

CONCLUSION

It can conclude that :

1. The number of viscosity and density from the microalgae doesn't fulfill the qualification of SNI that should be required in producing biodiesel.
2. The number of acid and saponification from the microalgae has fulfill the qualification of SNI that should be required in producing biodiesel.
3. The higher ratio of N-Hexane solvent resulting in lower viscosity rate (low fluid consistency). However, the determination of viscosity and density don't significantly give impact to the biodiesel formula wherefore it only shows the measure of a fluid's resistance to flow and the compressibility of a substance which doesn't affect the essence of the formula. Therefore, it needs further treatment to increase the number of viscosity and density.
4. The *Chlorella vulgaris* in the ratio of 1:4 and 1:8 both are effective as a base of biodiesel due to the qualification standard based on SNI of acid number and saponification number are fulfilled but the ratio of 1:8 is more effective to be the material for biodiesel than the ratio of 1:4 because it has higher ratio of acid number than the ratio of 1:4.

ACKNOWLEDGEMENT

For the first and foremost, we would like to express our deepest gratitude for the Almighty God, Allah SWT for the marvelous and amazing grace, for the countless blessings and love so we can finally complete this paper. We also like to

express our appreciation to the following people:
1. Yuda Setiabudi, S.T, our First Counselor and Paper Advisor that had given us a great guidance.
2. Yusuf Rahmad, S.Pd, our Second Paper Advisor that had given us information to improve our writing.
3. Ibrahim Azis, S.T; Dyah Wulandari, S.Psi; BrigjenMochamadMasrukin, M.Han; Ratna Dewi Budiati, our parents who have been there in helping, supporting and giving motivation to finish our paper.

REFERENCES

Li Xi, et all. (2021). Conversion of Pt nanoparticles encapsulated within MIL-101(Fe) to FePt intermetallic nanoparticles supported on carbon promotes formic acid electrooxidation. *Electrochemistry Communications 127 (2021) 107054.*

Pribyl Pavel, et all. (2021). Production of lipids in 10 strains of Chlorella and Parachlorella, and enhanced lipid productivity in Chlorella vulgaris. *Appl Microbiol Biotechnol (2012) 94:549–561*.

Khotimah Khusnul. (2018). MEMBANGUN KETAHANAN ENERGI PENDUKUNG PERTAHANAN MARITIM MELALUI PEMANFAATAN MIKROALGA SEBAGAI BIODIESEL BAGI MASYARAKAT PESISIR. *Appl Microbiol Biotechnol (2012) 94:549–561. Jurnal Pertahanan & Bela Negara | April 2018, Volume 8 Nomor 1.*

Gultom Sarman. (2018). Mikroalga: Sumber EnergiTerbarukan Masa Depan. JurnalKelautan Volume 11, No. 1.

Chisti Yusuf. (2007). Biodiesel from microalgae beats bioethanol. School of Engineering, Massey University, Private Bag 11 222, Palmerston North, New Zealand.

Chen Guanyi, et all. (2014). Chitosan and Its Derivatives Applied in Harvesting Microalgae for Biodiesel Production: An Outlook. Hindawi Publishing Corporation Journal of Nanomaterials Volume 2014, Article ID 217537, 9 pages.

Wati Adhik, et all. EKSTRAKSI MINYAK DARI MIKROALGA JENIS CHLORELLA sp BERBANTUKAN ULTRASONIK. Jurusan Teknik Kimia, Fakultas Teknik, Universitas Diponegoro.

Chao Hechun, et all. (2013). Direct Biodiesel Production from Wet Microalgae Biomass of Chlorella pyrenoidosa through In Situ Transesterification. Hindawi Publishing Corporation BioMed Research International Volume 2013, Article ID 930686, 6 pages.

Young Chul Lee, et all (2014). Recent nanoparticle engineering advances in microalgal cultivation and harvesting processes of biodiesel production: A review. Department of BioNano Technology, Gachon University, Seongnam-si, Gyeonggi-do 461-701, Republic of Korea.

Sarkar Rajesh Dev, et all (2021). Enhanced lipid accumulation in microalgae through nanoparticle-mediated approach, for biodiesel production: A mini-review. Department of Biotechnology, Gauhati University, Guwahati, Assam, 781014, India

Purwanto Edy, et all (2013). SINTESA BIODIESEL DARI MIKROALGA CHLORELLA VULGARIS MELALUI REAKSI TRANSESTERIFIKASI IN SITU. Jurusan Teknik Kimia, Fakultas Teknik. Universitas Surabaya.

Converti Attilio, et all (2009). Effect of temperature and nitrogen concentration on the growth and lipid content of Nannochloropsisoculata and Chlorella vulgaris for biodiesel production. Department of Chemical and Process Engineering "G.B. Bonino", University of Genoa, Via Opera Pia 15, 16145 Genoa, Italy.

Widjaja Arief, Chien Chao-Chang, Ju Yi-Hsu. (2009). Study of increasing lipid production from fresh water microalgae Chlorella vulgaris. Journal of the Taiwan Institute of Chemical Engineers 40 (2009) 13–20

Bidir Michael G, et all. (2021). The role of nanoparticles on biofuel production and as an additive in ternary blend fuelled diesel engine: A review. Energy Reports 7 (2021) 3614–3627.

Mauricio Tatiana, et all. (2023). Differences and Similarities in Lipid Composition, Nutritional Value, and Bioactive Potential of Four Edible Chlorella vulgaris Strains. Foods 2023, 12, 1625.

Choi Moon-Hee, et all. (2014). Aquatic ecotoxicity effect of engineered aminoclay nanoparticles. Ecotoxicology and Environmental Safety 102 (2014) 34–41.

Nam Hyungseok, Choi Julius, C. Capareda. (2016). Comparative study of vacuum and fractional distillation using pyrolytic microalgae (Nannochloropsisoculata) bio-oil. Algal Research 17 (2016) 87–96.