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Production and Characterization of Third-Generation Biodiesel from Microalgae Oil

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ABSTRACT

Third-generation biodiesel, derived from microalgae, presents a sustainable alternative to fossil fuels with no compromise on food security or utilization of large areas of arable land. This study deals with the production of biodiesel from *Chlorella vulgaris* microalgae oil by using a two-stage process, acid-catalyzed esterification followed by base-catalyzed transesterification, and the characterization of the produced biodiesel. Acid esterification of crude oil with 0.8 wt% H_2SO_4 lowered the acid value to 0.46 mg KOH/g, thereby meeting the quality requirements for subsequent transesterification. A maximum yield of biodiesel of about 94.86% was obtained through base-catalyzed transesterification under the reaction parameter of 9:1 methanol to oil molar ratio, 1.2 wt% KOH, 55°C reaction temperature, and 120 minutes reaction time. Characterization of the produced biodiesel and raw oil was performed, measuring density, kinematic viscosity, calorific value, and acid value according to ASTM standards. Overall, it satisfies the international biodiesel specifications given by ASTM D6751 and EN 14214. The results brought out the immense potential of *Chlorella vulgaris* as one of the most promising feedstocks for high-yield biodiesel production in the quest for sustainable energy.

Keywords: Microalgae Biodiesel, *Chlorella Vulgaris*, Transesterification.

1. Introduction

With the current increase in world energy demand, along with a decline in fossil fuel availability, much attention has been directed toward biofuels as an alternative energy source. Biodiesel represents one of the renewable alternatives that could stabilize or reduce dependence on conventional fossil fuels [1]. In addition, to meet the higher energy demand, biodiesel has also been considered one of the finest options to help mitigate greenhouse gas emissions and, consequently, deal with climate change [2]. However, it is evident that the transition from fossil fuels to biofuels would not be an easy process; there are considerable differences between the different generations of biofuels according to their advantages and limitations. Biodiesel, being a substitute for fossil fuel, was first derived from food crops, such as corn, sugarcane, and soybeans. This type of biodiesel is called 1st generation biodiesel. Large-scale 1st generation biodiesel production has received ethical and environmental criticisms due to its competition with food production, apart from using large stretches of land [3]. Food security became increasingly a cause for concern, and therefore, the emphasis started shifting toward the quest for more environmentally friendly alternatives. The disadvantages of the deficiencies of 1st generation biofuels were overcome in the development of second-generation biofuels using non-food biomass such as agricultural residues, wood chips, and waste materials [4]. These feedstocks are considered to be in direct competition with food crops; however, in general, the complex processing technologies for the conversion of lignocellulosic biomass to biofuels are costly and inefficient at large scale [5]. The high cost of conversion processes has so far prevented the widespread adoption of 2nd generation biofuels. Due to the disadvantages in both 1st generation and 2nd generation biofuels, attention has been drawn to 3rd generation biofuels, mainly produced from microalgae. Compared with terrestrial crops, Microalgae have several key advantages: fast growth rates, no requirement for arable land, and far higher potential lipid accumulation for bio-diesel conversion [6]. Besides, microalgae can capture CO_2 directly from industrial emissions and thus serve as a handy tool to reduce atmospheric CO_2 levels while producing energy [7]. Various species of microalgae, such as *Botryococcus braunii*, *Nannochloropsis sp.*, *Dunaliella primolecta*, *Chlorella sp.*, and *Crypthecodinium cohnii* provide large scale of lipid content ranging from 20% to 80% according to their dry biomass weight which are very compatible for high yield biodiesel production [8]. Depending on several parameters such as algae species and culture conditions, the dry weight of microalgae can provide more than 80% lipid content [9]. Lipid content of different generations of feedstock is listed in Table 1. Due to the significant advantages of microalgae, researchers have paid close attention to exploring the field of 3rd generation biodiesel. The selection of algal species is crucial for producing biodiesel, as it ensures low-cost culture media, high lipid content, and biomass productivity. Feng et al. cultured *Chlorella vulgaris* microalgae in artificial wastewater medium and achieved the lipid content of 42% and lipid productivity of 147 mg/l d^{-1} which represents a high potential candidate for synthesizing third generation biodiesel compared to other species. In addition, they presented a cost analysis and reported that *Chlorella vulgaris* algal biomass is a promising alternative to petroleum at $63.97 per barrel for wastewater treatment [10].

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Consequently, considering these advantages *Chlorella Vulgaris* microalgae species was selected as a third-generation feedstock for this study. Biodiesel production from *Spirulina maxima* microalgae was conducted by Rahman et al. [11] through two-step process. The first step was acid esterification which reduced the acid value (AV) of crude algal oil from 10.66 to 0.51 mg KOH/g oil. The second step was base transesterification to produce desired biodiesel, and they obtained maximum biodiesel yielding 86.1% in optimized reaction conditions, which are a methanol to oil molar ratio of 9:1, temperature of 65°C, magnetic stirrer speed of 600 rpm, base catalyst KOH concentration of 0.75 wt%, and a reaction time of 20 minutes. To improve process efficiency and obtain optimized parameters for the transesterification reaction, Annam et al. [13] incorporated Response Surface Methodology (RSM). They reported a maximum biodiesel yield of 83.54% from seaweed *Sargassum myriocystum*, which was predicted by RSM and gave the optimum reaction parameters of 120 min reaction time, 60°C temperature, 1:6 (v/v) oil to alcohol ratio, and 0.4 (w/w) catalyst amount.

Table 1 Lipid content of different feedstock. [12]

Generation	Feedstock	Lipid content (% dry weight biomass)
1st	Corn (Zea mays)	44
1st	Soybean (Glycine max)	18
1st	Sunflower (*Helianthus annuus*)	41
1st	Palm oil (*Elaeis guineensis*)	40
2nd	Jatropha (*Jatropha curcas*)	28
2nd	Camelina (*Camelina sativa*)	42
3rd	*Phorphyridium cruentum*	9-14
3rd	*Pavlova salina*	12-30
3rd	*Phaeodactylum tricornutum*	18-57
3rd	*Chlamydomonas sp.*	21-27
3rd	*Scenedesmus obliquus*	30-50
3rd	*Chlorella sp.*	28-53

Additionally, Garg et al. [14] showed a comparison between RSM and ANN models in process parameter optimization for biodiesel production. The R^2 values for RSM and Artificial Neural Network (ANN) were 0.9657 and 0.999651 respectively which indicated that ANN model was more accurate than RSM. Microalgae derived biodiesel provide better emission quality than conventional diesel in compression ignition (CI) engine [15]. Rajak et al. [16] conducted the diesel engine performance and emission analyzing test with six different blends of Spirulina microalgae biodiesel. They found reduction in brake thermal efficiency (BTE) by 0.98%, exhaust gas temperature (EGT) by 1.7%, HC by 16.3%, CO by 3.6%, NO_X emission by of 6.8%, and smoke emission by 12.35% and an increase in brake specific fuel consumption (BSFC) by up to 5.2% and CO2 emission by 2.8% for biodiesel blend (B20%) as compared to diesel (B0%) at full load condition engine with constant engine speed 1500 rpm.

The above studies depict that biodiesel from microalgae is a promising alternative to fossil fuels. However, production of third-generation biodiesel from Chlorella Vulgaris microalgae is still in the developing stage. To increase exposure to the role of *Chlorella Vulgaris* microalgae, the objectives of this study are to produce and characterize the key properties of biodiesel derived from *Chlorella Vulgaris* microalgae oil (CVMO) through a two-stage process (acid-catalyzed esterification and base-catalyzed transesterification). A comparison of fuel properties has also been shown between *Chlorella Vulgaris* microalgae biodiesel (CVMB) and conventional diesel.

2. Materials and Method

2.1 Materials

Algal oil of *Chlorella Vulgaris* was purchased from Liable Essential Oil Products Private Limited, UP, India. The reagents, including methanol (CH_3OH) (99.9% purity), anhydrous sulfuric acid (H_2SO_4) (purity > 98%), and potassium hydroxide (KOH) pellets (purity 99%) were used in this study and sourced from the local market of Chattogram, Bangladesh.

2.2 Experimental Set-up

Whatman filter paper was used in this study for filtering the biodiesel. Two 250 ml, three-necked round-bottom reactor flasks were used to conduct the experiments (esterification and transesterification). The three-necked reactor flask with a flat bottom water bath (capacity: 2 L) was placed on a magnetic stirrer hot plate (Model: DLAB MS7 H550 Pro) at the Metallurgy Engineering Laboratory in CUET to conduct the experiment. The reactor was equipped with a magnetic stirrer hot plate and one external temperature sensor (Model: PT1000). The temperature sensor was inserted into one neck of the reactor using a rubber seal.

2.3 Production of Biodiesel

Fig. 1 illustrates the step-by-step workflow of the biodiesel production process used in this study.

2.3.1 Filtering

Pre-treatment was performed to overcome difficulties, such as contamination and undesired soap formation, caused by the higher AV content, leading to a decline in biodiesel yield during the transesterification reaction. The pre-treatment process includes refining the raw oil by filtering through filter paper and acid-based esterification to reduce AV. First, the raw oil sample was pre-heated up to 105^{o}C for 30 minutes to eliminate the unwanted contaminants and water content before starting the esterification reaction and filtered through filter paper.

2.3.2 Acid-Catalyzed Esterification

In this step, 25 grams of filtered CVMO was taken into 3-neck flat bottom conical flask to heat in proper temperature. The 3-neck flat bottom conical flask was kept merged in a water bath for appropriate heating. Then 8.2 grams (methanol to oil molar ratio 9:1) of methanol was mixed with the heated oil. When the temperature was stable, 0.2 gram (0.8 wt%) of sulfuric acid was added dropwise carefully. Rigorous stirring was provided by a magnetic stirrer at 780 rpm mixing speed, creating proper turbulence in the reactor. The reaction parameters of acid-catalyzed esterification are mentioned in Table 2. After the reaction

was complete, the esterified oil (EO) was poured into a separatory funnel to separate the excess alcohol, free fatty acids (FFAs), and impurities. Under these conditions, the product was kept for approximately 4 hours. The excess alcohol, H_2SO_4, and the FFAs were observed in the top layer. In contrast, the esterified product with a low acid value (AV) was located in the bottom layer of the separatory funnel.

2.3.3 Titration

The EO was separated from the top layer of the funnel for AV analysis by titration using phenolphthalein as an indicator. The AV of esterified oil was found 0.46 mg KOH/g oil, which met the satisfactory condition (acid value < 1 mg KOH/g oil) [17]. Then, transesterification was conducted to convert the EO into high-yield biodiesel. Acid value was calculated using Eq. (1).

$$\boldsymbol{Acid\ value} = \frac{\boldsymbol{V \times N \times 56.1}}{\boldsymbol{W}} \quad (1)$$

Here, V and N indicate the volume(ml) of KOH required to neutralize the acid and the normality of KOH, respectively. In addition, W means the mass of sample oil.

2.3.4 Base-Catalyzed Transesterification

The transesterification was conducted for biodiesel synthesis. The reaction was performed employing the EO and 0.3 gram (0.3 wt%) of KOH was applied as a catalyst. With the pellets of KOH and 8.2 grams (methanol to oil molar ratio 9:1) of CH_3OH, the solution of potassium methoxide (CH_3OK) was made in a conical flask, and the solution was mixed until the pellets of KOH fully dissolved. The EO was heated up to a desirable temperature for about 30 minutes to remove any undesirable constituents, then the heated solution of CH_3OK was applied to the esterified sample to start the reaction for the synthesis of biodiesel. Several researchers conducted transesterification reactions at various reaction rates, molar ratios, catalyst loadings, reaction times, and temperatures to achieve the maximum biodiesel yield [18]. The reaction parameters of base-catalyzed transesterification and achieved yield of biodiesel are mentioned in Table 2.

Table 2 Reaction parameters of producing biodiesel.

Reaction Parameter	Esterification	Transesterification
Methano : Oil (molar ratio)	9:1	9:1
Catalyst (wt%)	0.8	1.2
Temperature (oC)	60	55
Mixing Speed (rpm)	780	900
Time (min)	60	120

2.3.5 Biodiesel Separation

As soon as the transesterification was finished, the mixture was sent to a separatory funnel for allowing to separate glycerol and raw biodiesel. The mixture was kept for settling for about 7-8 hours at room temperature. After separation, there were two phases, with a clear, dark-colored glycerol at the bottom of the flask and raw biodiesel at the top. The reason is that, density of biodiesel is lower than glycerol. The color of the biodiesel depends on the feedstock oil used. The lower layer of glycerol was drained off and upper layer was proceeded for washing and drying.

2.3.6 Washing and Drying

The crude biodiesel contains excess methanol, potassium hydroxide, and other impurities. The separated biodiesel was washed with 50% (v/v) hot distilled water of 40°C-50°C temperature in spray method to remove the undesired impurities. This consecutive hot water spray washing process was performed several (3-5) times up to pH of 4.5. After that, the washed biodiesel was heated up to 100°C for 2-3 minutes to vaporize the water and impurities. Finally, the pure biodiesel was obtained.

2.4 Characterization of Biodiesel

Several properties of CVMO and CVMB have been measured following ASTM methods and are listed in the following Table 3.

2.4.1 Density

Density is a property of how much mass is occupied within a given volume. The fuel density is critical to the injector nozzle, as it influences engine operation. This can also impact the efficiency of fuel atomization in airless combustion systems. Biodiesel typically has a higher density and lower compressibility than diesel fuel. The density of CVMO and CVMB was determined using the ASTM D891 test method, with a 50 ml glass pycnometer at 40°C temperature.

2.4.2 Kinematic Viscosity

Viscosity is a property of fluids that causes a resistance force to act on them, resulting in gradual deformation under shear forces. The viscosity of fuel increases at low temperatures, which affects the liquidity of the fuel. In this study, the kinematic viscosity of CVMO and CVMB was determined according to ASTM D7042, using a rotating-cylinder viscometer.

2.4.3 Calorific Value

The heating value or calorific value is one of the most prominent parameters for characterizing a fuel because of its energy content. The calorific values of CVMO and CVMB were determined in accordance with ASTM D240 using a bomb calorimeter. The sample was collected in a crucible within a bomb calorimeter. Before the experiment started, the combination's weight was determined. To ensure that the magnesium wire comes into contact with the mixture, the crucible must be set above a ring. In the bomb calorimeter, with oxygen present, the mixture burns at 25 atm. The calorimeter's internal temperature rises as the fuel sample burns. We calculated the calorific value of the mixture's unit mass after determining the temperature differential between the internal and exterior water.

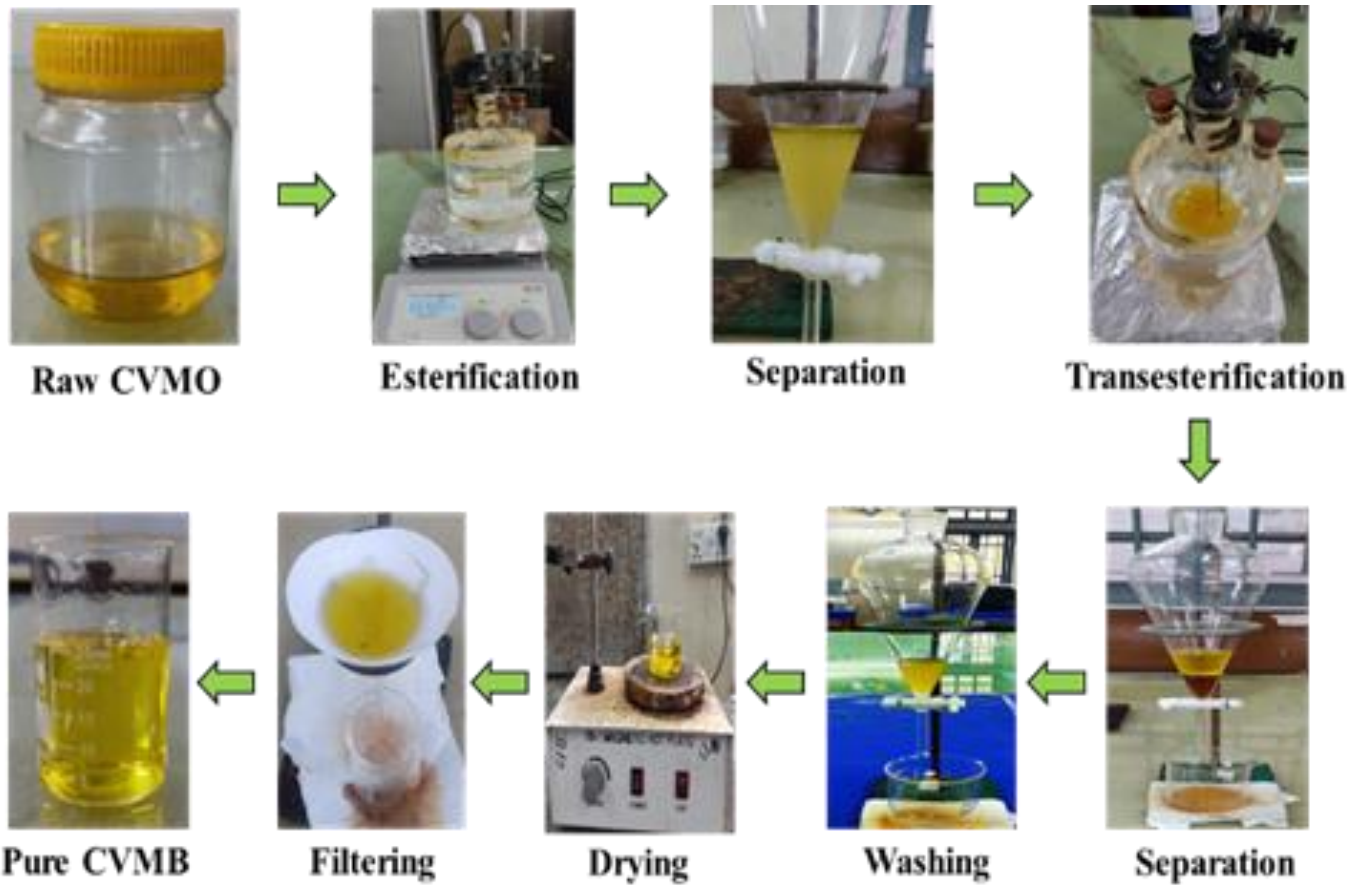

Fig. 1 Biodiesel production stages.

2.4.4 Acid Value

The content of FFAs in a pure fuel is known as AV or neutralization number. Additionally, the significance of AV is the amount of KOH (mg) required for neutralizing 1g of the sample. FFAs have a proportional relationship with AV. In this study, we evaluated the AV of CVMO and CVMB according to ASTM D974, using phenolphthalein as an indicator and KOH solution as the titrant.

Table 3 Properties of CVMO and CVMB.

Properties	Method	CVMO	CVMB
Density at 40oC (kg/m^3)	ASTM D891	896.4	865.2
Kinematic Viscosity at 40oC (cSt)	ASTM D7042	28.43	5.1
Calorific Value (MJ/kg)	ASTM D240	31.83	40
Acid Value (mg KOH/g Oil)	ASTM D974	1.2	0.45

3. Result and Discussion

3.1 Biodiesel Yield

Biodiesel yield refers to the ratio between raw oil and purified biodiesel. We achieved 94.86% biodiesel yield from Chlorella Vulgaris microalgae oil undergoing a two-step route. First one was acid esterification step, where FFAs were effectively converted to methyl esters. For this reason, AV has been reduced which prevent unwanted soap formation. The following step was base-catalyzed transesterification, which broke triglycerides into three fatty acid methyl ester (FAME) molecules and byproduct glycerol. In this step, we used the reaction parameter as 9:1 methanol to oil molar ratio, high mixing speed 900 rpm, suitable reaction temperature 55ºC, catalyst 1.2 wt%, and reaction time 120 minutes. Furthermore, proper separation, washing and drying were done to remove excessive byproduct and moisture which enabled the comparatively high yield [19]. Eq. (2) was used to calculate the biodiesel yield.

$$Biodiesel\ Yield = \frac{mass\ of\ pure\ biodiesel}{mass\ of\ the\ raw\ oil} \times 100\% \quad (2)$$

3.2 Properties of Biodiesel

The production CVMB from CVMO showed an optimal shift in properties toward conventional diesel-like behavior. In the case of density, we observed a 4.0% increase in CVMB relative to petro-diesel. Biodiesel typically has a higher density and lower compressibility than diesel fuel [20]. The inclusion of unsaturated acids with more than two double bonds increases the density of biodiesel. However, base-catalyzed trans-esterification reaction makes a 3.5% reduction in the density of biodiesel relative to the CVMO by separating the glycerol as a byproduct. The average density of CVMB (865.2 kg/m^3 at 40ºC) is a bit lower than the 872.5 kg/m^3 documented by Rashd et al. for biodiesel obtained from *Scenedesmus parvus* microalgae using esterification and trans- esterification process, suggesting that our *Chlorella vulgaris* biodiesel is consistent with the conventional density range of algal biodiesel [21]. Fig. 2 shows the density comparison.

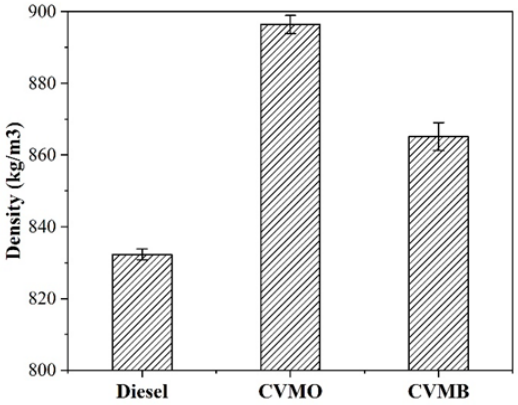

Fig. 2 Comparison of density among diesel, CVMO and CVMB (Error bars represent standard deviation)

Kinematic viscosity is the most eminent physico-chemical parameter of biodiesel, as it has an impact on the function of fuel injection equipment along with spray atomization. After transesterification, we found an 82% reduction of kinematic viscosity from CVMO to CVMB. The reason is that one large triglyceride (TAG) molecule in oil is converted into three smaller fatty acid methyl ester (FAME) molecules and glycerol [22]. This drop has a positive impact on fuel atomization and spray penetration in diesel engine combustion. With an average kinematic viscosity of 5.1 cSt, CVMB shows an analogous value to the 5.2 cSt experimented by Hariram et al. for *Dunaliella salina* microalgae biodiesel [23]. Fig. 3 represents the comparison in kinematic viscosity.

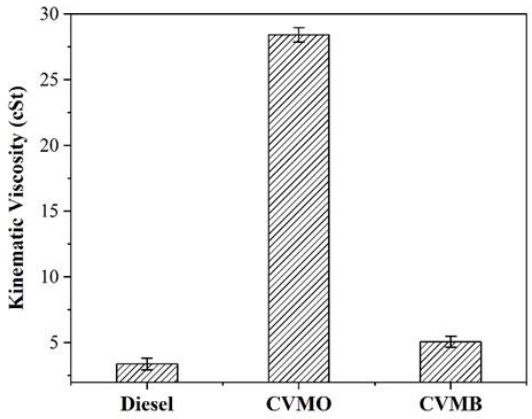

Fig. 3 Comparison of kinematic viscosity among diesel, CVMO and CVMB (Error bars represent standard deviation)

For calorific value, CVMB is 13.5% lower than diesel. Although a higher calorific value is desired for diesel engine combustion, biodiesel has lower calorific values than diesel due to its higher oxygen content [24]. Moreover, a higher oxygen content promotes complete combustion of biodiesel in the engine. The measured average calorific value (40 MJ/kg) of CVMB is almost similar with *Dunaliella salina* microalgae biodiesel, which was synthesized by Hariram et al. using transesterification method [23]. Fig. 4 depicts the comparison of calorific value.

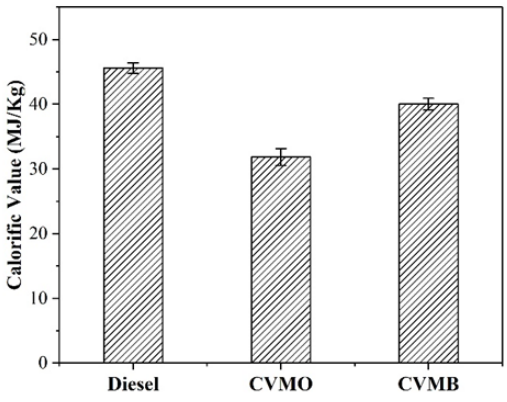

Fig. 4 Comparison of calorific value among diesel, CVMO and CVMB (Error bars represent standard deviation)

After the transesterification reaction, the acid value of CVMB drops to 0.46 mg KOH/g, which is a 58% reduction compared to CVMO. Reduced AV contains lower acid content in fuel which protects the fuel system of diesel engine from severe corrosion [25]. The average acid value of CVMB almost aligned with Ulva lactuca microalgae biodiesel, Yameen et al. used a novel catalyst (Cu–BTC@AC) to produce microalgae biodiesel with the help of transesterification [26]. Fig. 5 shows the comparison in acid value.

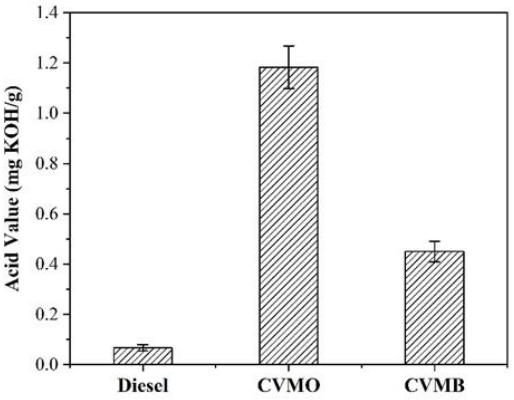

Fig. 5 Comparison of acid value with diesel, CVMO and CVMB (Error bars represent standard deviation)

4. Conclusion

The third-generation biodiesel produced from *Chlorella Vulgaris* microalgae oil has the potential to overcome the modern market fuel demand. In this study, a two-step method consisting of esterification and trans-esterification was performed, using methanol as the standard reagent, H_2SO_4 as the acid catalyst, and KOH as the base catalyst to produce biodiesel from CVMO. The first step, esterification, was performed to reduce the acid value in CVMO, which leads to unwanted soap formation during biodiesel washing and drastically reduces the biodiesel yield. In addition, the second step, transesterification, was done to synthesize biodiesel. Implementing suitable reaction parameters, this study successfully produced microalgae biodiesel, achieving a high yield of 94.86% which is in good alignment with both ASTM and EN biodiesel standards. In future studies, the optimal biodiesel yield could be achieved by combining RSM and ANN to optimize the process parameters. Another promising feedstock, genetically modified microalgae, is still under research, which is known as fourth-generation biodiesel.

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