Original Research Article

AN ASSESSMENT OF BIODIESEL PRODUCTION FROM THREE SPECIES OF MICRO ALGAE (*Chlamydomonas reinhardtii, Chroococcus* species and *Ankistrodesmus falcatus*)

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ABSTRACT

Aim: Over dependence on fossil fuels has triggered environmental and economic concerns, creating an ultimate need to redirect towards renewable energy options. Hence, the study on biodiesel production from algal biomass.
Study design: Modified open pond culture system was applied in the biomass culture and growth was monitored via pH and turbidity.
Place and Duration of Study: Study was done in the laboratory of the Department of Environmental Management and Toxicology, Federal University of Petroleum Resources, Effurun between June 2020 and July 2021.
Methodology: Soxhlet extraction was applied in algal oil extraction. Alkali-mediated transesterification of algal oil blends into biodiesel was conducted. Biodiesel blends were characterized physically and spectroscopically for fuel properties and chemical position. The synthesis and fuel properties of biodiesels from three micro algal species: *Chroococcus sp*, *Ankistrodesmus falcatus*, and *Chlamydomonas reinhardtii* were conducted. Due to low algal oil yield, characterization of four types of hybrid oils were prepared: 100 percen(%)t lavender oil (100LO), 10% *Ankistrodesmus falcatus oil* plus 90% LO (10AN90LO), 10% *Chlamydomonas reinhardtii* oil plus 90% LO (10CHL90LO), and 10% *Chroococcus sp* oil plus 90% LO (10CHR90LO).
Results: The synthesis and fuel properties of biodiesels from the micro algal species: *Chroococcus sp*, *Ankistrodesmus falcatus*, and *Chlamydomonas reinhardtii* gave a percentage yield of algal oil of 29.6%, 23.4% and 15.5%, respectively. The percentage yield of biodiesel from 10AN90LO*,* 10CHL90LO*,* 10CHL90LO was 66.7%, 61.7% and 50.0%, respectively. Gas Chromatography-Mass Spectrometry(GC-MS) analysis revealed presence of four fatty acid methyl and vinyl esters namely; hexadecanoic acid methyl ester, octadecadienoic acid methyl ester, methyl stearate, carbonic acid eicosyl vinyl ester and carbonic acid, tetradecyl vinyl ester. Fourier transform infrared spectroscopy(FTIS) confirms that chemicals produced are esters.
Conclusion: Fuel properties of biodiesel from these selected algal strains appear appreciable when compared to standard limits. Blends with petroleum diesel showed great potential for use in diesel engines.

*Keywords: M*icroalgae, biodiesel, hybrid oils, transesterification, characterization

1. INTRODUCTION

Due to a continuous rise in energy demands coupled with economic and environmental factors, there comes the need to develop technologies that will utilize

energy from renewable sources [1]. It is imperative for us to explore alternate fuel supplies especially the non-fossil fuel energy sector. An example of such is biofuel technology. Biofuels are non-toxic biodegradable fuels which can be synthesized chemically or biologically from algae, plants' or animals' oils and fats as well as used or recycled oils and fats [2]. Researches on biofuels are mainly on biomass and waste and has evolved over the years [3]. They are the first generation biofuels. They are those produced from food-based crops, and correspond mainly to ethanol-based fuels (obtained from the fermentation of sugar based crops like corn, beet, sugarcane etc.), vegetable oil-based fuels (i.e. biodiesel, raw oil, and renewable diesel produced from catalytic hydro-deoxygenation) from oleaginous plants (oil rich plants like colza, palm, canola, etc.) and biogas emitted from raw material or landfills [3]. Second generation biofuels are the cellulosic-based biofuels obtained from non-food crops materials (wood, leaves, straw, etc.). These biofuels include bio-alcohols, bio-oil, 2,5-dimethylfuran (BioDMF), bio-hydrogen, and wood diesel [4]. Third generation biofuels are microorganisms' (yeast, fungi) biofuels and algae-based fuels like vegetable oils, bio-oil, jet-fuels, bio-hydrogen, biodiesel, renewable diesel and many others [5]. There are several biofuel options in production today, one of which is biodiesel.

Biodiesel is any diesel-equivalent fuel made up of renewable biological materials like plant oils or livestock fats [6]. Biodiesel is a generic term for a variety of ester-based oxygenated fuels derived from renewable biological sources [7]. Transesterification is the process of converting long-chained triglycerides in oils into fatty acid methyl esters (FAME) called biodiesel. It is labeled as B100 and must comply with ASTM D 6751 [8]. Biodiesel can be made from any oil or lipid source of which the major components of these sources are triglycerides molecules. In the presence of organic or inorganic catalysts, these triglycerides are converted to different groups of fatty acid methyl esters called biodiesel. Feedstock for biodiesel are pure vegetable oil, animal fats, waste cooking oil, waste motor oil and algal oil. Though most are suitable for biodiesel production, they are food based raw materials and contribute to the food chain. Their utilization can lead to increase in price and scarcity of these feedstocks [9]. To address these challenges, research is focused on exploration, development and utilization of dedicated energy crops which will be distinct from food for biodiesel production.

Microalgae are potential feedstock [10]. Microalgae are a group of autotrophic eukaryotes with simple cellular structures which can be unicellular or multicellular. They manufacture and store energy in the form of lipids and other energy sources. It has attracted appreciable attention from researchers, entrepreneurs and governments as an alternative non-food biodiesel feedstock owing to the low production cost, high oil yield and rapid biomass production [9]. It does not compete with any current human interests and offer many environmental benefits. Such as carbon dioxide bioremediation, for energy production, do not require agricultural land, can be found and grown anywhere, grow quickly compared to terrestrial crops and as fertilizer.

There are various methods of production and processing of algae oil. Culturing of microalgae can be through open ponds or enclosed photo-bioreactors. Open ponds method uses a shallow pond about a foot deep, and the algae are raised in circumstances that are similar to those found in their native habitat. The algal cells and nutrients in the water body are mixed and circulated by a paddle wheel in a raceway design. Despite the fact that open ponds are less costly to build and operate, they have their own set of disadvantages when compared to enclosed photo-bioreactors. Open-air systems commonly lose a significant amount of water due to evaporation.

Due to this, open ponds reduce biomass production because microalgae cannot use carbon dioxide as effectively as they otherwise could in these environments [11].
In the enclosed photo-bioreactors, transparent materials are used for these devices, which are often placed outside in the open to let in natural light. The culture vessels' surface area to volume ratio is high. The most common type of photo-bioreactor is a tubular device with a number of transparent tubes facing the sun's rays. On the other hand, enclosed photo-bioreactors have a number of shortcomings. Microalgae growth is restricted by temperature and light intensity variations even though enclosed systems can increase biomass concentration [12].
Harvesting include gravity settlement and use of centrifuge. Extraction processes are mechanical extraction, solvent extraction, traditional extraction and supercritical fluid extraction [13]. Methods of biodiesel synthesis include; physical method, chemical method and biological method [2]. In addition, characterization of oil feedstock for biodiesel production and biodiesel produced is necessary. Characterization help in the determination of the quality of the oil used and biodiesel produced [14]. Examples of such for the oil feedstock are free fatty acid (FFA) and acid value, saponification value, color, specific gravity and others. For the biodiesel produced characterization conducted include pour point, melt point, oxidative stability, iodine value, flash point etc.
Biofuel is being considered as an alternate energy source due to the resultant depletion of petroleum-derived fuel and environmental concerns, Therefore, steady substitution of biofuel for diesel can reduce the consumption of petroleum resources while also reducing the extreme climate change caused by automobile pollution. The environmental benefits of algal biofuels over fossil fuels was the push behind this research with focus on biodiesel production from these three micro algal species: *Ankistrodesmus falcatus*, *Chroococcus sp* and *Chlamydomonas reinhardtii*.

2. MATERIALS AND METHODS

2.1 Selection of algal strains

Three micro algal species; *Ankistrodesmus falcatus, Chroococus sp*, and *Chlamydomonas reinhardtii* were used in the study. A pure culture of each species was obtained from the Department of Plant Biology and Biotechnology, University of Benin, Benin-city, Edo State. The cultures were kept in an ice chest and transported to the laboratory. Modified Chu's Medium No.10 solution was used to cultivate the algal species [15]. The algal cultures were stabilized for three days after being exposed to mild sunshine for 16 hours.
The culture method employed was a modified open pond culture system, in which three microalgae species: *Ankistrodesmus falcatus, Chroococus sp,* and *Chlamydomonas reinhardtii* were cultivated separately in unsealed 100mls plastic containers (vessels). Using micro-element, macro-element, iron stock, and vitamin stock solution as documented by [15]. Each species growth culture was left for twelve (12) weeks with continual stirring. The pH and turbidity of the culture were measured to track the algal growth. Weekly, a turbidity meter was used to measure the turbidity of an aliquot of each species from the different vessel.

2.2 Harvesting and drying

After 12 weeks, the cultures reached a stationary phase, and the broth were collected into separate containers. To precipitate the algal cells, a flocculant, aluminum sulfate anhydrous $Al_2(S0_4)^3$, was added to the broth at a concentration of 1g/L (1g of $Al_2(S0_4)^3$ per liter of algal broth). The contents of the containers were manually swirled to allow algal cells to bind with the $Al_2(S0_4)^3$, then allowed to settle into two layers over night for total precipitation. The top transparent layer was gently decanted, leaving a highly dense cell solution residue at the bottom. The residue was then manually filtered to create an algal paste. Depending on the water content, the algal paste was spread on a tray and allowed to air dry at room temperature for an average of seven days. Beginning on day 6 after air-drying, the algal biomass was weighed and reweighed every day until a consistent weight was attained. The dried algal biomass was then collected in dry jars that had been pre-weighed. After filling with dried algal biomass, the jars were weighed again [15]. Equation (1) was used to calculate the mass of dried algal biomass.

$$(\textbf{Jar + dry biomass}) - (\textbf{empty jar}) = \textbf{mass of algal dry biomass [g]} \quad \text{... (1)}$$

Equation 2 was used to get the final concentration of algal broth.

$$\frac{(\textbf{massofalgaldrybiomass [g]})}{(\textbf{volumeofculturemedium [L]})} = \textbf{final concentration [g of dry algae/L]} \quad \text{... (2)}$$

2.3 Extraction of algal oil

Using a soxhlet system, lipids were extracted from dried algal biomass. Based on the number of algae species accessible for lipid extraction, the soxhlet extractor was set up three times. Lipids were isolated from the three algae species (*Ankistrodesmus falcatus, Chroococcus sp,* and *Chlamydomonas reinhardtii*). The oil was extracted from the algal biomass using 95% pure normal hexane (n-hexane). 250 mL n-hexane was transferred to a 500 mL round bottom flask that had been pre-weighed. The flask was linked to a Soxhlet extractor using glass wool to prevent algal biomass from clogging the solvent line or falling into the solvent flask (n-hexane). On top of the glass-wool, 91g, 88g, and 76g of algal biomass were applied, respectively. Thereafter, the extractor was linked to the condenser, which circulated cold water from a running faucet. The extraction process was left for around 6 hours after the flask was heated by a heating mantle, and the sample compartment was practically colorless, indicating the end of the extraction. The flask containing the algal oil in hexane was allowed to cool at room temperature when the extraction was completed. To eliminate algal particles from the oil/hexane mixture, the contents of the flask were filtered through 0.25m Whatman filter paper grade number 5 using a Buchner funnel and flask. To eliminate surplus moisture from the sample (mixture of algae oil/hexane), 3g of sodium sulfate (Na_2SO_4) crystals was used as a drying agent. Before the filtering process, the Na_2SO_4 crystals were poured to the round bottom flask and gently stirred for about three (3) minutes. Afterwards, the filtrate was placed in a water bath to evaporate any remaining solvents from the algal oil extracts.

Equation 3 was used to compute the % output of algal oil.

$$\% \text{ output of algal oil} = \frac{[(\textbf{massof (algaloil+flask)} - \textbf{massof (emptyflask)}]\textbf{gX100})}{(\textbf{massof (dryalgalbiomassweight)g}} \quad \text{.....3}$$

2.4 Preparation of algal oil for biodiesel production

The oil output was low, and the extracted algal oil had a high free fatty acid (FFA) value. The methods of [16] and [17] were adopted as their reports showed low yield. When all of the extraction solvent had evaporated from the algal oils, the algal oil solidified as expected. A proportion of extraction solvent was left with the algal oils in order to retain them in a liquid form. Due to the low volume of algae oil, the three species of algal oils were blended separately with lavender oil (LO) at a ratio of 1:9, suggesting that 10mls of each algae oil were combined with 90mls of lavender oil. Pure lavender oil was also tested concurrently but separately as a control experiment to account for any potential inconsistencies. For characterization, four types of hybrid oils were prepared: 100% lavender oil (100LO), 10% *Ankistrodesmus falcatus* plus 90 percent LO (10AN90LO), 10% *Chlamydomonas reinhardtii* plus 90 percent LO (10CHL90LO), and 10% *Chroococcus sp* plus 90 percent LO (10CHR90LO).

2.5 Algal oil identification

The ASTM standard was used to characterize the innovative fuel oils (100LO, 10AN90LO, 10CHL90LO, and 10CHR90LO). To guarantee their eligibility for biodiesel production, fuel parameters such as color description, specific gravity, kinematic viscosity, acid value, free fatty acid (FFA), saponification value, and ester value were examined.

2.6 Production and blending of biodiesel

The production of biodiesel from three different micro algal species was carried out in accordance with a small modification of the protocols specified by [18]. After separating the extracting solvent (n-hexane) from the algal oil, 60mls of the algae oil mix from *Ankistrodesmus falcatus* were measured and placed into a 250 ml conical flask, which was heated to 50°C. In a 250ml beaker, about 0.6g of potassium hydroxide (KOH) pellet was measured (i.e., catalyst concentration of 0.6 percent wt). In the 250 mL beaker, 13.1 mL of methanol was added to make a potassium methoxide solution (i.e. mole ratio of oil to methanol of 1:6). *Chroococcus sp*, *Chlamydomonas reinhardtii* and lavender non-edible oil were all treated with 12.9ml, 13.2ml, and 12.6ml, respectively of methanol. The solution was thoroughly agitated until the potassium hydroxide pellet was completely dissolved to make potassium methoxide solution (methanolic KOH). This was heated to 60°C in the oven before pouring into the warm algal oil and stirring with a magnetic stirrer. The mixture was then constantly whirled under reflux for 50 minutes at a temperature of 50°C. The mixture was allowed to cool and settle for 24 hours in a separatory funnel. The biodiesel was left in its impure state after the lower contents were emptied. The biodiesel was then washed in warm water to eliminate any remaining glycerol and soap in the funnel. This was repeated until clear water was visible beneath the biodiesel in the separatory funnel, which was then drained. After that, 5g of anhydrous sodium sulfate, a drying agent, was added to the biodiesel, which was spun for 3

minutes to remove any remaining water and Watmann filter paper was used to filter it. The excess solvent in the transesterified sample was then evaporated in a water bath at 40°C for two hours. The finished product was ready to be characterized. The amount of biodiesel that was gathered was measured and documented. Using equation 4, the percentage yield of biodiesel was estimated.
Biodiesel yield percentage = mass of biodiesel produced multiplied by 100... (4)

2.7 Mixture of methyl esters from algal oil species

To blend methyl esters from algal oil species with ordinary diesel fuel, the splash method was used (BO). *Ankistrodesmus falcatus, Chroococcus sp, Chlamydomonas reinhardtii*, and lavender oil in four different blends: BO, B10, B20, and B100. B0 (pure petroleum diesel), B10 (10 percent biodiesel and 90 percent petroleum diesel), B20 (20 percent biodiesel and 80 percent petroleum diesel), and B100 (pure biodiesel) were all tested physicochemically and spectroscopically. The decision to combine in such a proportion was made for the goal of optimizing a simulated motor engine that could run on biodiesel.

2.8 Biodiesel and blends characterization

The biodiesel blends were tested for physicochemical (fuel) qualities in accordance with ASTM standards, with certain changes. Kinematic viscosity at 37.8^0C, specific gravity and API gravity at 16^0C, fire point, cloud point, oxidation stability, water content, and flash point were all determined. The spectrophotometric characterization of produced biodiesel and blends was carried out using gas chromatography mass spectroscopy (GC-MS) and fourier-transform infrared spectroscopy (FTIR).

3. RESULTS AND DISCUSSION

3.1 Turbidity and pH

Organic matter such as planktons, algae, and other microscopic creatures, as well as some soluble organic and inorganic matter cause turbidity in water [19]. While the turbidity values for *Chlamydomonas reinhardtii, Ankistrodesmus falcatus,* and *Chroococcus* species ranged from 10 to 91, 4 to 87, and 9 to 137 NTU respectively. The pH values rose from 4.5 to 9.7, 4.8 to 9.4, and 4.9 to 8.9 for *Chlamydomonas reinhardtii, Ankistrodesmus falcatus,* and *Chroococcus* species, respectively. The pH and turbidity of the broth appeared to be proportional to the algal cell concentration based on the results (Fig.1 and Fig.2, respectively). This was in line with the findings of [20] as increased number of algal cells in the culture medium reduced its transparency.

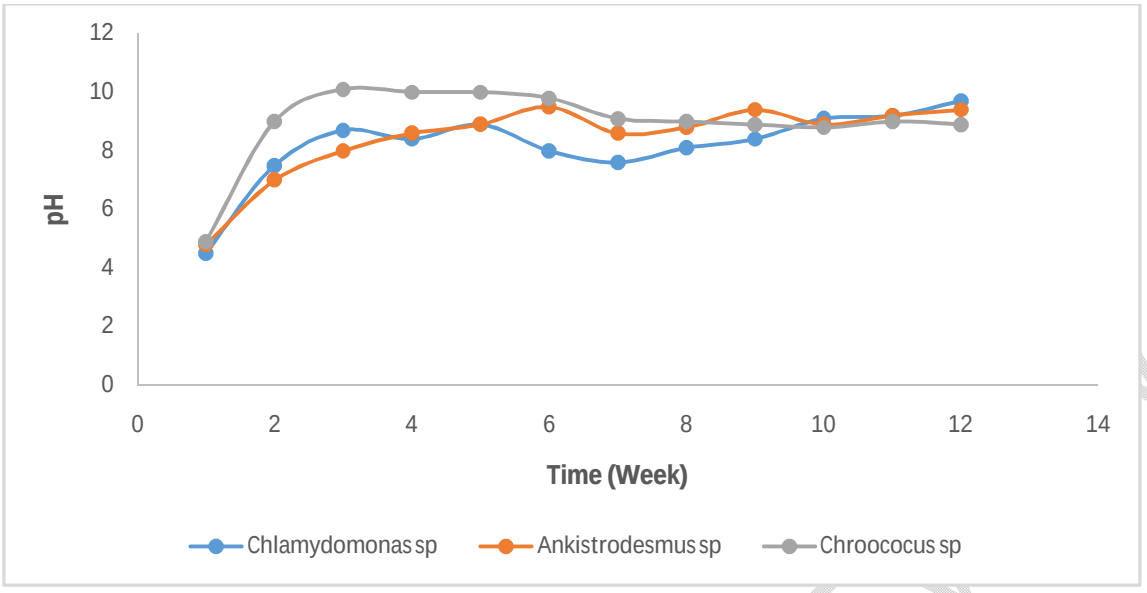

Fig.!: pH changes during 12 weeks of culture

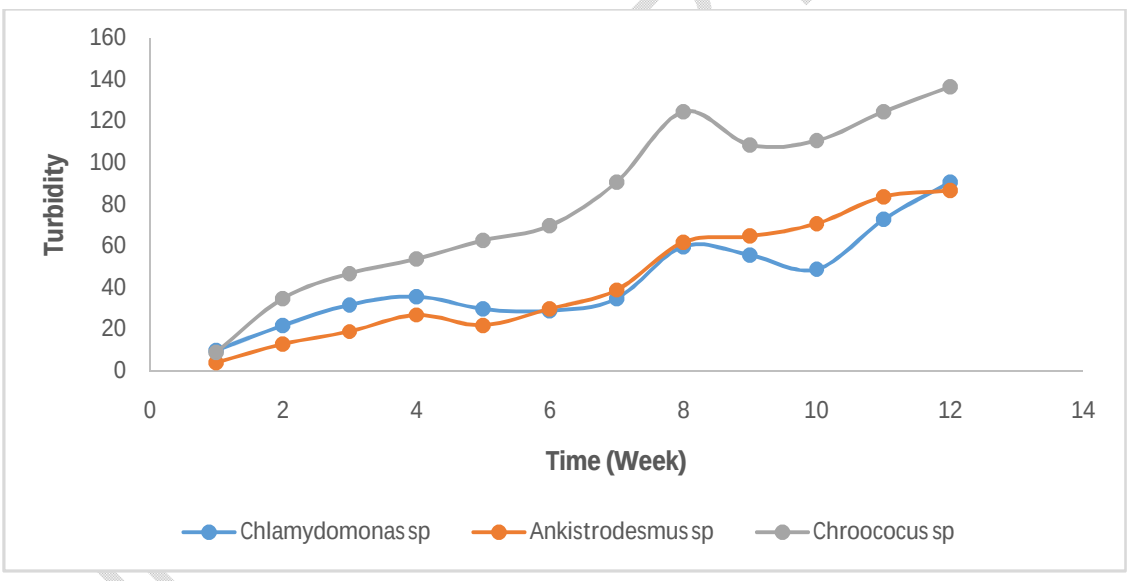

Fig.2: Turbidity of the culture during 12 weeks

3.2 Percentage oil yield of algal species

Different values of percentage yield were produced from the extraction of algal oils from each strain of algae. Tables 1 and 2 show the raw masses (in grams) obtained, as well as the percentage yield for algal oils and biodiesel respectively for all the strains of algae. Table 1 highlights the percentage oil yield of the three microalgae species. The biodiesel yields from 10AN90LO, 10CHL90LO, and 10CHR90LO are shown in Table 2. From Table 1, the total biomass yielded rather low amounts of algal oil. Kumar *et al*. [17] reported a similar low yield in their reports. As a result, each of the three algal oils was blended separately with lavender oil, a non-edible vegetable oil, in 1:9 ratios, resulting in 10mls of each algae oil and 90mls of lavender non-edible oil. It's worth noting that once all of the extraction solvent evaporated from the algal oil, it hardened. A small amount of extraction solvent was left with the oils in order to maintain them in liquid state. Pure lavender oil was evaluated concurrently but separately as a control to make up for any possible inconsistencies. Table 2 shows a relatively high percentage output of biodiesel from blends, which may be owing in part to the blended lavender oil.

Table 1: Percentage yield of algal oils*

Algal Strain	Volume of Culture Medium (L)	Average mass of dry biomass (g)	final concentration of algal broth (g/L)	Average mass of oil extract (g)	Percentage yield of oil (%)
Ankistrodesmus falcatus	100	91±1.02	0.91±0.01	21.31±0.24	23.4±0.26
Chroococcus sp	100	88±0.99	0.88±0.01	26.09±0.30	29.6±0.33
Chlamydomonas reinhardtii	100	76 ±1.95	0.76±0.02	11.81±0.37	15.5±0.41

*Values are mean ± standard deviation of triplicate determinations.

Table 2: Percentage yield of biodiesel blend at 10:90 algae oil and Lavender blend*

Algal Strain	Average mass of blend	Average mass of biodiesel	Percentage yield of biodiesel
Ankistrodesmus falcatus	60±0.85	40±0.56	66.7±0.94
Chroococcus sp.	60±0.98	30±0.49	50.0±0.82
Chlamydomonas reinhardtii	60±0.59	37±0.37	61.7±0.63
Lavender**	60±0.67	50±0.56	83.3±0.94

*Values are mean ± standard deviation of triplicate determinations.
**Biodiesel was also synthesized from pure Lavender oil without blending, which served as a control.

3.3 Physicochemical parameters of oils and biodiesel blends

Table 3 shows the physicochemical tests performed on the algal oil to determine its eligibility for biodiesel synthesis in terms of color, physical state, specific gravities, and kinematic viscosities, algal oil blends showed promising qualities for biodiesel synthesis. However, most oil blends have quite high acid values, with the exception of lavender, which is followed by *Chroococcus sp*. Lavender oil appears to be better appropriate for biodiesel synthesis when these values are compared to their saponification values. Despite the fact that FFA has been linked to lower biodiesel yields [21], its removal via alkali pretreatment is usually required when other methods than alkali-mediated transesterification are used. The importance of algal oil's physicochemical qualities was to determine its suitability for use in biodiesel manufacturing. Akubugwo *et al*. [22] did a comparative examination of vegetable oils for home and industrial purposes and found that physicochemical criteria are also required to assess the nutritional value or otherwise of the oils.
The physicochemical parameters measured on biodiesel-pure diesel blends is displayed in Table 4. Some physicochemical properties of biodiesel blends were within the specified range for biodiesel. Except for biodiesel from *Chlamydomonas reinhardtii*, which showed a high degree of oxidative stability, pure biodiesel (B100) from each of the algal oils appeared to be relatively unstable (239 minutes). The oxidative stability of algal biodiesel and petro-diesel (B10 and B20) was improved

when they were blended. This could be related to the high amount of saturation in petro diesel imparted to biodiesel as a result of mixing. Unsaturation, among other qualities, enhances the oxidative properties of biodiesel, lowering its stability, according to a review by [23]. It's worth noting that biodiesel and blends derived from *Chlamydomonas reinhardtii* had the maximum oxidative stability, whereas lavender blends had the lowest. It may thus be deduced that, despite its high biodiesel yield, pure biodiesel from lavender is exceedingly unstable, with an oxidative stability of only 98 minutes, well below predicted limits (180 minutes). This means that pure biodiesel from lavender, as well as those from *Ankistrodesmus falcatus* and *Chroococcus sp,* are prone to oxidative rancidity, with the exception of *Chlamydomonas reinhardtii*, which has a good oxidative stability (B100 =239 minutes).

The pour points of biodiesel mixes are within expected ranges of -15 to 10oC, with most pure (unblended) biodiesel values being higher than mixed biodiesel values. Pure lavender (B100) appears to have a greater pour point than its related blends (B10 and B20 lavender), with a value of -7.8 (B100) compared to -10.3 and -10.1 for B10 and B20, respectively. Similarly, as seen from values beyond the limitations, the cloud points of biodiesel blends demonstrate substantial results (Table 4). Nonetheless, these pour and cloud point values show that these blends have a lot of potential for application in temperate climates and cold seasons without danger of clogging pipes, containers and engines because they all fall within normal ranges [24]. Except for *Chroococcus* species whose B10 flash point of 89^0C is slightly out of range (93.0^oC), the flash points of blends are largely within range. The addition of petro-diesel does, however, appear to alter the flash points of blends, albeit in a minor way. This is as observed by the considerable drop in flash point values of their respective B10 and B20 blends when compared to their B100 counterparts [18].

The most essential biodiesel characteristics were also established including specific gravity, API gravity, and kinematic viscosity (Table 4). All of the blends' specific gravities were within acceptable limits. Blends have different effects for the other two crucial physicochemical criteria, as can be seen. For example, all biodiesel blends deviated from the usual API gravity range of 29 to 35, and there was no discernible pattern between the blended and unblended biodiesel values. Percentage mixing is expected to have a positive or negative impact on physicochemical characteristics, as evidenced by other test results. However, deviations from the approved range may be overlooked for two reasons. First, their specific gravities are all in the same ballpark, and second, the deviation is little and may be addressed with more statistical analysis. It's worth noting that the kinematic viscosity values for all 100 percent biodiesel (B100) was outside the range (1.9-6.0), however when mixed with petro diesel, the kinematic viscosity values increased significantly. Water content varies for the different blends.

Table 3. Physicochemical parameters of oil blends

Property (units)	Test Method	Lavender	*Ankistrodesmus falcatus*	*Chlamydomonas reinhardtii*	*Chroococcus sp.*
Blending ratio (Algal oil:Lavender)	-	0:100	10:90	10:90	10:90
Total Volume of sample (cm^3)	-	100	100	100	100
State	Visual	Liquid	Liquid	Liquid	Liquid
Colour description	Visual	Light yellow	Dark brown	Dark brown	Dark brown
Specific gravity @ 37^oC	(ASTM D287)	0.8796±0.0021	0.9266±0.0037	0.9012±0.0032	0.8998±0.0028
Kinematic Viscosity @ 37^oC in cSt	(ASTM D1298, D445)	1.29±0.00	1.89±0.01	1.81±0.01	1.76±0.01
Acid value (mg KOH/g)	(ASTM D664)	0.8417±0.0020	1.0100±0.0040	1.0661±0.0038	0.9539±0.0030
Free Fatty Acids (%)	(ASTM D482)	0.4208±0.0010	0.5050±0.0070	0.5330±0.0067	0.4769±0.0045
Saponification value (mg KOH/g of oil)	(ASTM D94)	184.00±1.43	192.1424±1.49	193.545±1.51	189.3380±1.47
Ester Value (mg KOH/g)	(ASTM D1617)	183.1583±1.42	191.1325±1.47	192.4789±1.50	188.3841±1.44

*Values are mean ± standard deviation of triplicate determinations.

Table 4. Physicochemical parameters of biodiesel – pure diesel blends

Property (unit)	Test Method	Limit / range	Diesel Sample												
			Pure Petro-Diesel	*Ankistrodesmus falcatus*			*Chroococus sp.*			*Chlamydomonas reinhardtii*			Lavender		
Blend %			**B0**	**B10**	**B20**	**B100**	**B10**	**B20**	**B100**	**B10**	**B20**	**B100**	**B10**	**B20**	**B100**
Oxidation Stability (minutes)	**EN 14112**	≥180	497±4.11	363±3.00	363±3.10	131±1.08	389±3.22	385±3.18	122±1.01	395±3.27	387±3.20	239±2.43	355±2.94	309±2.56	98±0.82
Cloud Point (^{o}C)	**ASTM D2500**	-3 to 12	-9.8± 0.08	-9.3± 0.07	-8.3± 0.07	-6.4± 0.07	-8.5± 0.06	-8.2± 0.09	-5.3± 0.04	-7.8± 0.07	-7.7± 0.16	-5.9± 0.77	-8.1± 0.16	-7.6± 0.45	-4.2± 0.41
Pour Point (^{o}C)	**ASTM D2500**	-15 to 10	-12.8±	-12.5±	-12.6±	-11.6±	-12.2±	-11.2±	-10.4±	-12.7±	-11.9±	-11.2±	-10.3±	-10.1±	-7.8±
Flash Point (^{o}C)	**ASTM D93**	≥93.0	79±0.8	98±0.8	98±1.1	111± 2.4	89±0.7	101± 1.9	98±1.0	96±0.9	102± 3.1	100± 3.9	99± 5.2	109± 5.5	145± 4.3
Calorific Value (MJ/Kg)	**ASTM D240**	40.2± 4	43.696 ±0.362	38.788 ±0.321	41.788 ±0.346	31.407 ±0.260	40.504 ±0.335	40.218 ±0.333	36.075 ±0.299	40.154 ±0.332	40.035 ±0.331	36.554 ±0.303	40.042 ±0.334	39.371 ±0.326	29.769 ±0.247
Specific gravity @ 16^{o}C/60^{o}F	**(ASTM 1298)**	0.85-0.88	0.8511 ±0.009	0.8579 ±0.010	0.8615 ±0.006	0.8631 ±0.005	0.8588 ±0.004	0.8619 ±0.008	0.8652 ±0.002	0.8601 ±0.007	0.8600 ±0.001	0.8640 ±0.011	0.8598 ±0.000	0.8611 ±0.002	0.8620 ±0.012
API gravity @ 16^{o}C/60^{o}F	**(ASTM D287)**	29-35	38.75± 3.93	37.37± 2.29	36.65± 1.25	36.36± 1.27	37.19± 2.35	36.57± 1.50	37.96± 2.90	36.93± 1.59	36.95± 1.66	36.16± 1.75	36.99± 1.21	36.73± 1.32	36.55± 1.35
Kinematic Viscosity @ 37.8^{o}C in cSt	**(ASTM 446)**	1.9-6.0	2.63± 0.03	2.99± 0.11	3.32± 0.53	7.69± 1.22	2.95± 0.09	3.25± 0.98	8.01± 2.89	3.11± 0.49	3.39± 0.67	7.85± 2.01	3.38± 0.47	3.49± 1.33	6.34± 2.22
Water Content (%)	**(ASTM 2709)**	0.050 max	0.01± 0.00	0.03± 0.00	0.03± 0.00	0.02± 0.00	0.03± 0.01	0.02± 0.01	0.02± 0.00	0.02± 0.00	0.02± 0.00	0.03± 0.01	0.03± 0.00	0.01± 0.00	0.02± 0.01

3.4 GC-MS profiling of biodiesel – pure diesel blends

GC-MS analysis was used to evaluate the percentage composition of fatty acid methyl esters (FAME) present in biodiesels and blends. Peak regions (percent peak area) of the chromatogram, as revealed by their unique retention time, showed the percent composition of each component in the sample (Table 5).

Table 5 shows that biodiesels from all samples had four FAMEs in common. Hexadecanoic acid, methyl ester, 9,12-Octadecadienoic acid (Z,Z)-, methyl ester, 9-Octadecenoic acid (Z)-, methyl ester, and Methyl stearate are the FAMEs in question. While *Chroococcus* species*, Chlamydomonas reinhardtii*, and Lavender all have carbonic acid, eicosyl vinyl ester, only Lavender has carbonic acid, tetradecyl vinyl ester. It's worth noting that all *Ankistrodesmus falcatus* components were determined to be FAME. Other components were found common to *Chroococcus* species, *Chlamydomonas reinhardtii* and Lavender. Specifically, 1-Octadecene, Tetracosane and Octadecane are other major components common to both *Chroococcus* species and *Chlamydomonas reinhardtii* while Nonadecene is common to both *Chlamydomonas* and Lavender and Tricosane is common to the trio of *Chroococcus* species, *Chlamydomonas reinhardtii* and Lavender. Percentage composition of FAME in biodiesel was found to be highest in *Ankistrodesmus falcatus* (100%), and lowest in lavender (76.20%). Several other components and their respective percentage composition are displayed in Table 5 and their variations in percentage composition could be partly related to reaction conditions and catalyst during transesterification, as well as nature of triglycerides present in oil blends before transesterification.

Table 5: Chemical composition of FAME in biodiesels (B100) from Gas Chromatogram

Compound	Percentage Composition (Peak Area (%))				
	Retention Time	*Ankistrodesmus falcatus* Biodiesel Blend B100	*Chroococcus sp.* Biodiesel Blend B100	*Chlamydomonas reihardtii* Biodiesel Blend B100	Lavender Biodiesel Blend B100
Fatty Acid Methyl Esters					
Hexadecanoic acid, methyl ester	15.796	13.68	7.74	9.12	4.47
9,12-Octadecadienoic acid (Z,Z)-, methyl ester	18.107	59.42	30.72	40.70	26.60
9-Octadecenoic acid (Z)-, methyl ester	18.218	19.69	23.49	22.10	28.52
Methyl stearate	18.541	7.21	5.19	5.65	2.90
Carbonic acid, eicosyl vinyl ester	19.596	-	10.30	6.69	10.60
Carbonic acid, tetradecyl vinyl ester	12.986	-	-	-	3.11
Other Major Components					
1-Octadecene	16.930	-	2.31	4.40	-
Nonadecene	22.240	-	-	2.46	1.92
Tricosane	22.051	-	3.29	2.51	6.84
Tetracosane	23.151	-	3.51	3.12	-
Octadecane	17.396	-	4.07	2.46	-
Others	-	-	9.38	0.79	15.04
Total	-	100	100	100	100
% Composition of FAME in Biodiesel	-	100	77.44	84.26	76.20

3.5 FT-IR Characterization of Biodiesel blends

The FT-IR characterization results for each FAME from biodiesels indicate a similar trend of a strong intense carbonyl (C=O) stretch near 1735cm-1, which is typical of an ester. The lack of an acidic O-H stretch near 3400cm-1, as well as an amide N-H stretch, confirmed this to a large extent. The presence of a strong, intense C-O bend between 1300 and 1000cm-1 confirmed that the chemicals produced are esters. Other IR bends include a faint C=C peak around 1680-1620cm-1 (indicating unsaturation in the compound) and an aliphatic C-H stretch around 2800cm-1 (indicating unsaturation in the compound). CH_2 peaks (around 1500cm-1), a long chain band (about 700cm-1), and a C-C band are among the other peaks in the fingerprint regions (near 1100cm-1).

4. CONCLUSION

Biodiesels derived from *Ankistrodesmus falcatus, Chroococus* species*, Chlamydomonas reinhaditii* oils show potentials as a biofuel and may be used to replace fossil diesel under most operating conditions, with little or no engine changes. *Chlamydomonas sp* has the highest potential as it produced the highest quality of biodiesel when compared to those from the other two algal species studied. Also the biodiesel blends with pure diesel is useful as well, as it will help to reduce dependence on only fossil fuel diesel.This will ameliorate the environmental impacts caused by the use of pure fossil produced diesel.

Further studies to genetically manipulate algal species, particularly *Chlamydomonas reinhardtii*, in order to increase the oil quality of biomass as it produced the highest quality of biodiesel when compared to the other two algae species studied, would be of great importance. Building local reactors to include a whole conversion process from algae culture to biodiesel in the presence of biocatalysts and other environmentally acceptable components is a potential field to explore. Storage facilities and additives that might extend the life of biodiesel without compromising the fuel's integrity should be investigated. Finally, more studies on the use of various cost-effective and environmentally friendly materials (biological catalyst) for biodiesel production should be encouraged.

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