Renewable Energy from the Seaweed Chlorella Pyrenoidosa Cultivated in Developed Systems

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Abstract- The purpose of this present study is to demonstrate the potentialities of the alga *Chlorella pyrenoidosa* as a source of energy of third generation, based on the increase of biomass yield, by the use of systems of culture developed, these latter are doped by are doped with optically active molecules, allowing for shifting the light to the area of photosynthesis adapted to the alga, what increasing the maximum biomass concentration compared to the neutral system (non-doped) chosen as a reference by a factor of 2 from 3th days of culture. We have also, realized a simple extraction of the oil of cultivated alga, the yield acquired is interesting, it is of the order of 40.17%, this oil undergoes a conversion to a biodiesel by the reaction of transesterification, which gives us a yield of 92.56%. In effect, based on the results above, this work leads to the conclusion that, the alga *Chlorella pyrenoidosa* rich in lipids ,constitutes a choice dilicate for the operate as raw material for the production of energy that is sustainable and renouvlabe.

Keywords Algae, Biomass, Biodiesel, *Chlorella pyrenoidosa*, Renewable energy, Transesterification.

1. Introduction

The sources of renewable energy respectful of the environment have taken a considerable place in the course of these last years, replacing the fossil fuel resources associated with negative factors ; which ignites the search to rethink to another source respectful of the environment and sustainable development [01].

Currently, to respond to these challenges, research tends toward the study of use of food products such as raw materials the main for the production of biodiesel and'ethanol. However, the exploitation of oleaginous plants for the production of biofuels, is in competition with the food products; which directs the research toward another ideal alternative source energy, which coming from the microalgae [01].

Historically, the use of vegetable oils as fuels, was conducted in 1893 by R. Diesel, who has worked for the first test of' an engine to the peanut oil. Then, the demand for bio-fuels was rather low due to low prices; then the interest of 'use of bio-diesel relaunched during the energy crises of the second world war and also of the crises in the years 1970- 1980. However, during the last decades and in several countries the problem of bio-diesel to completely change and their production has significantly increased [02].

At the moment, the request for liquid fuels for transport, continues to increase in view the concerns of the climate change, as well as the air pollution and the destruction of ecosystems, which are directly related to the production and combustion of oil [03]. In addition to these problems, the sources of conventional energies are not sustainable and their derivatives carry in the long-term with health and environmental consequences like the greenhouse effect, acid rain, as well as the pollutions atmospheric and the water [04]. Similarly, the use of the related products derived from petroleum products, such as chemicals and plastics, which are not biodegradable emit toxic fumes [05]. However, the rate of exhaustion of fossil fuels, created an interest for the bio-diesel [06], which has attracted the intensive attention as a important biofuel [07]; the biodiesel, a possible alternative that could replace fossil fuels and can become the first source of energy in the world [08, 09].

The bio-diesel presents several advantages compared to the diesel, such as it is bio-degradable, renewable, characterized by better lubrication, Sulfur in zero or very low, ecological, the emission of CO_2 is strongly reduced, with an appreciable reduction of some of the pollutants emitted and not dangerous to manipulate saw that it is non-toxic [10]. Chemically, the biodiesel is a mixture of alkylic esters of fatty acids, it is obtained by alcoholysis of triglycerides, in the presence of a catalyst [11], the methanol is generally the alcohol the most used, other alcohols such as : the ethanol and the alcohols C3 To C5, linear or ramified can be also used [12]. The transetérification by the basic catalysis, is the process most commonly applied, in order to convert an oil or fat with an alcohol to give the biodiesel , this type of basic catalysis in the advantage that It is much faster than the acid catalysis ; bases such as : NaOH or KOH have commonly been used as catalysts for this kined of reaction [13]. The oils used in the reaction of trans esterification are vegetable origin or animal fat [14], for the lipid extraction and especially those of microalgae, several methods are used: mechanical and chemical [15], The extraction is accelerated by the chemical solvents [16] ; The effectiveness of the extraction of oil increases by the use of solvents such as methanol, chloroform and the n- hexane, this last, which is a non-polar solvent, was largely used in the extraction of vegetable oils [17].

In the purpose of the realization of a renewable and sustainable energy balance, various food materials such as cereals, oilseeds, sugar cane and same residues are currently being exploited as load of primary primary feedstock and are used in the production of the ethanol, of agrochemical products and biofuels including biodiesel [18, 19]. The biodiesel produced from oleaginous plants, would create a lack of the vegetable oil, intended essentially for human consumption [20]. Although the oil oilseed crops cultures are renewable resources, the production of biodiesel from these cultures, was found to be unsustainable [21]. Indeed, an increasing criticism concerns the sustainability of many first-generation biofuels, new trends are stimulated the interest to develop other second generation biofuels, which are produced from non-food feedstocks, such as lignocellulose [07]. But several obstacles imposed, such as considerations of space, low efficiency of the production of lipids and long periods of culture, and other major obstacles not technical must be resolved for the industrialization and the commercialization of bio-diesel of the agricultural sectors and traditional foresters [10]. However, the agrroalimentary residues are very necessary in the cycle of natural replenishment of the soils, their eliminations in continues will affect the fertility of the soil, or even the erosion of soils. Moreover, the risk of sensitization for the food security, which can put pressure on the global socio-economic stability especially for economically weak regions [14].

Therefore, by taking account these factors, micro-algae are used for the production of fat as alternative to the agriculture and the animals the sources.

Recently, the development of bioenergy has been highlighted, using the microalgae as biomass of 3rd generation [22, 23]. The use of these microorganisms such as raw materials for biofuels, received a considerable attention because of their various advantages compared to the other compared to other organisms and higher plants [07]. These photosynthetic microorganisms, capable of converting the sun light, the water and carbon dioxide in sugars, producing biological macromolecules, such as lipids [24] ; Note that there is a variety of these photosynthetic microorganisms able to fix CO_2 from the atmosphere more effectively and quickly that the terrestrial plants [25] ; The typical composition in fatty acids of an oil of microalgae, is mainly constituted of a mixture of unsaturated fatty acids (Palmitoleic, oleic, linoleic and linolenic acid) and saturated fatty acids: palmitic and stearic acids are also present in low quantity [26].

Otherwise, the micoalgues receive an increasing and particular attention throughout the world, for their exploitation as bio-fuels, view their rapid growth rate, their ability to accumulate high amounts of lipids in their cells [27, 28, 29]. In fact, several species of this group are able to accumulate high amounts in lipids and can be a load suitable feedstock for producing biodiesel [10], This lipid content can be reached up to 80% in optimal conditions of growth, as well as their yields in biomass and oils (lipids) are ten times greater than those recorded for terrestrial plants [30, 31, 32]. According to M. Mubarak and al the microalgae can accumulate lipids similar to vegetable oils, their production potential of oil is 100 times higher per acre of land compared with any other plants, of this effect, the biofuels produced from algae are the fuels more appropriate for the future [33].

Chlorella, is a unicellular green algae of freshwater [34, 35], widely used as a food supplement for health in various countries [36], their high content in antioxidants to reserve an important place for the use in the food and medicinal fileds [37, 38]. The *Chlorella* alga held the attention of biotechnologists, it is considered as an important source of the Biomass [39], it is one of the promoteuses algae for the production of biodiesel, due to their high content in lipids which varies from 20 to 30% [40].

In this article, we present an overview of the appearance of the third generation biodiesel, this study focused specifically on increasing biomass yield and therefore, the increase in biodiesel yield, the basic idea consists to cultivate the chosen alga in developed systems, the latter are doped by optically active molecules which allows the movement of the waves of the light towards the zone of photosynthesis, this in order to get more biomass in a minimum of time, focusing on the choice of the microalga *Chlorella pyrenoidosa*. In this present work, the extraction of oil and the transesterification are also discussed. It should also be noted that, some characteristics namely : pH, viscosity, density and the index of refraction are determined for the extracted oil and for the synthesized biodisel, this last is characterized also by IR spectrophotometry.

2. Materials and Methods

2. 1. Culture of alga Chlorella pyrenoidosa

The stumps of the alga *Chlorella pyrenoidosa* were grown in seven systems (plastic tubes PMMA, diameter 1 cm length 50 cm), the first system is neutral and chosen as a reference, while the others are doped with different concentrations of optically active componds, in a way to have a cascade effect, to move the quantity of light at low wavelength toward the area of the photosynthesis for the alga.

These cultures are carried out at the laboratory (Fig. 1) under an artificial light provided by three neon lamps of 18 W/m^2, the temperature varies from 25°C to 27°C, the agitation is provided by a small air pump, allowing the homogeneous distribution of the air in the cultures, As well, we have used the same nutrient medium for all cultures, which is the middle of the Bold (1967) modified with pH = 6.9. The followed of the alga growth is carried out by measuring the absorbance at 680 nm by a UV spectrophotometer.

Fig. 1. Photo of the experience.

2. 2. Oil extraction

The extraction in the Soxhlet is a technique commonly practiced, it allows to realize continuous extractions solid - liquid using cycles of vaporization - condensation of the solvent. This method presents the advantage of being simple to use [41] and reduces the solvent consumption [15].

The extractor "Soxhlet" enables the processing of solid with solvents in the liquid phase. The body of the extractor, containing a cartridge filled solid and attach on a solvent reservoir and surmounted by a refrigerant. The solvent is vaporized then condensed and remains in contact with the solid. The solution is drained periodically by the priming a siphon. The solution of the balloon grows rich gradually by solute and the solid is always put in contact with the solvent.

In this present study, the strains are retrieved from the culture systems, dried in the open air dry in the open air away from the light, in order to use as raw material to the extraction by soxhlet.

We filled the cartridge with a quantity of seaweed prepared and put on the support of device Soxhet, afterwards, it has been added 150 mL of solvent n- Hexane in the balloon and to place it in the support ; this operation lasts several cycles and at the end of extraction on test by a plate CCM and a UV lamp, after the operation of the extraction pass on to the stage of the evaporation to obtain the oil extract and recover the solvent.

*2. 3. Biodiesel synthesis*The methyl ester are obtained by the reaction of trans-esterification of tri-glycerides of oil obtained previously, in our case we used the methanol as a reagent for the reaction and the hudroxyde of sodium as catalyst. The synthesis of bio-diesel involves four essential steps :

The first one is the reaction, the practical realization of the reaction of trans-esterification is carried out in a simple mounting at reflux, we dissolved the catalyst in the methanol, the mixture is stirred and is heated very slightly, pours the resulting solution in the oil and shake vigorously and after two hours of agitation pour the mixture into a separatory funnel and separated the biodiesel.

The second step is the decantation, which values the success of a reaction of trans-esterification, it is materialized by the presence of two phases. The glycerol whose the greater density than the ester is located at the bottom of the bulb has decanted after several hours of settling.

The third step is the washing, the obtained bio-diesel must be washed to eliminate the excess of alcohol and catalyst. Place the bio-diesel in a separatory funnel and slowly pour 10 mL of water for rinsing. This operation is delicate, it should be done very gently with the least agitation possible because the agitation causes the formation of an emulsion which decreases the yield of the synthesis. Leave again decant approximately 24 hours then recover the bio-diesel. The last step, the purification, which consists in recovering the bio-diesel and we add 1 to 2 % of its mass of calcium chloride ($CaCl_2$) to absorb the humidity that has remained and finally filter and retrieve the bio-diesel.

2. Results and Discussion

3. 1. Culture of Chlorella pyrenoidosa

3. 1. 1. Biomass evolution

Fig. 2 presents the evolution of biomass depending on temples, during the experience :

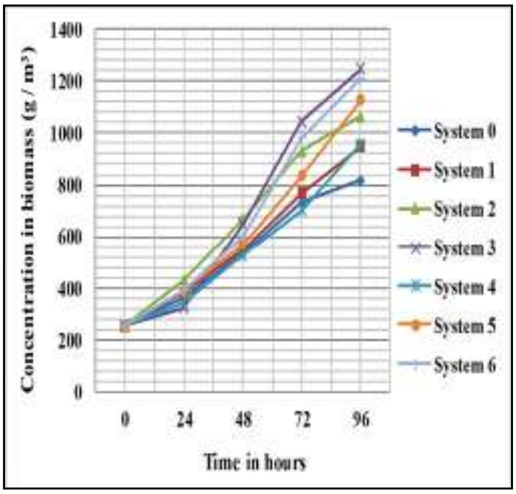

Fig. 2. Evolution of the biomass.

According to the results obtained of the Fig.2., it is clear that, the concentrations in biomass of cultures performed in the doped systems are higher than that of the system not doped chosen as a reference, we also note that the pace of the curves are identical with a small gap of a system to the other, the maximum value in the biomass is registered for the third system. While, the lowest concentration is obtained for the system N°4 which is 959,7 g/m^3 after 96 hours of cultivation. On another side, the results show that the concentration in biomass obtained for all the systems is greater than 500 g/m^3 in the first two days, a growth is recorded from third days and the maximum concentration was increased by a factor of 2. This may be due exclusively to spectral shifts of the light, generated by the optically activate molecules involved.

For all doped systems, we observe that the concentration in biomass remains to increase, while the culture realized in the neutral system, the strains grown in these systems are in their exponential phases, the strains cultured in these systems reached their stationary phase from 5 th day of culture, this can be justified by the positive effect of spectral shift, which appears very important for the cultures of microalgae under low luminous intensity.

Knowing that, the systems used in this study are doped by different concentrations in optically active namely: Lumogen Yellow, Rhodamine B, Rhodamine 8G and 2.5-diphenyloxazole OPP, the systems are doped by a way has to have an cascade effect to move the quantity of light has weak wavelength towards the region of photosynthesis for the seaweed, molecules are chosen by such a kind as, the emission of 2,5-diphenyloxazole which is 460 nm corresponds of the excitement of Lumogen Jaune towards 450nm, the latter emits in the wavelength of excitement of Rhodamine B which is 540 nm, this element emits by its tour in the wavelength of excitement of Rhodamine 8G towards 625 nm. Indeed, further to the analyses of the obtained results which show very clearly that the shiffting spectral took place with effect signifcatife on the yield in biomass of seaweed *Chlorella pyrenoidosa*, what presents an interesting advantage, for the employment of this kind of the systems for the culture of seaweeds realized under low light, allowing of éconmiser the light énérgie as well as the time of culture.

3. 1. 2. The kinetics of growth

Specific growth rate was calculated by the following equation:

$$\mu = \frac{\ln C - \ln C_0}{t - t_0} \tag{1}$$

where C and C_0 are concentrations of biomass at the end (t) and the beginning (t_0) respectively of a cycle of treatment.

The growth rate results are shown in the following figure:

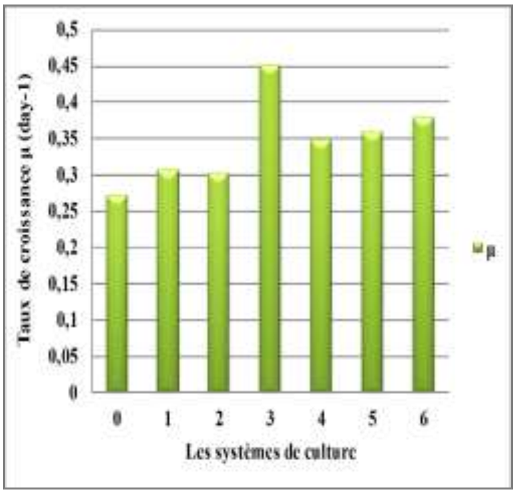

Fig. 3. The growth rate.

The analysis of the results presented in Fig.3. shows that, for all doped systems of cultures, the specific growth rate is higher than that recorded for the reference system (system 0), the maximum value is obtained for the system N=°3 with a rate of 0.4521 J^{-1}, it is higher to that of system 0 by a percentage of 65.69. This high output from system 3 (Fig.3 and Table 3) is exclusively due for the differences of the concentrations of molecules luminescent doped in these sytemes.

The totality of the specific growth rate for the doped systems used for the culture of the microalga *Chlorella pyrenoidosa*, are higher than the value of system not doped by different percentages, these latter are grouped in the following table :

Table. 1. Growth rates percentages.

Systems of culture	Percentage rate growth
System 0 (reference)	-
System 1	12.19788

System 2	10.60875
System 3	65.69201
System 4	27.43861
System 5	31.49807
System 6	38.94623

3. 2. Oil extraction
3. 2. 1. Extraction yield

Once the extracted oil was obtained, their yield was determined relative to the initial quantity of dry alga, according to the following formula:

$$\eta = \left(\frac{m_{extracted\ oil}}{m_{dry\ alga}}\right) \times 100 \quad (2)$$

With : η : the yield and m : the mass.
Practiclly, the yield obtained is:

$$\eta = \left(\frac{7.8708}{19.5938}\right) \times 100$$

$$\eta = 40.17\ \%$$

The lipid content of some kind of *Chlorella* microalgae species are presented in table 2 below:

Table. 2. Lipid content of some species of microalgae *Chlorella* kind

Algae species	Fat content (% dry weight)
Chlorella emersonii	25 - 63
Chlorella protothecoides	14.6 – 57.8
Chlorella sorokiniana	19 - 22
Chlorella vulgaris	5 - 58
Chlorella pyrenoidosa	2
Chlorella sp.	10 - 48

The comparison of yield achieved in our case which is 40.17 % with those of micro-algae kined *Chlorella* presented in table 2 above, shows us that this yield is very acceptable, note that according Mata and al [42] the values are variable between 2% obtained for *Chlorella pyrenoidosa* to 63 % for *Chlorella emersonii*. Indeed, the yield of *Chlorella pyrenoidosa* is only 2%, which presents the lowest value of the yields of the micro-algae kind *Chlorella*; this analysis tells us that our output is important, which indicates a very significant increase. This allows us to say that the spectral shift is a method of choice has applied in order to improve algae yields and consequently the increase of yield of oil.

3. 2. 2. Extracted oil characteristics

The determined characteristics of the oil extracted are: the density, viscosity, pH and the index of refraction. The results obtained are grouped in the following table :

Table. 3. Oil characteristics.

Parameter	Measured value
Cinematic viscosityy *(ν)*	*37.1120 Cp*
Density (d^{20})	*0.8714*
pH	*4.35*
Refractive index	*1.4532*

The analysis of the results of table 3 above shows that the extracted oil from the alga *Chlorella pyrenoidosa* have a density 0.8714 is lower by comparison with other oils, such as Peanut oil whose density is 0.921, Soya oil has a density of 0.923, Sunflower oil 0.924 and Rapeseed oil 0.92 [43].

As regards viscosity, our oil extracted with a kinematic viscosity of 37.1120 Cp at 28.5 ° C, Shows that it is higher for the use of this oil as a biofuel.

The extracted oil is characterized by a pH of the order of 4.35, which indicates that it is more acidic, it also characterized by a refractive index of 1.4532, which is less than that obtained for sunflower oil whose the refractive range: 1.4740 to 1.4760 at 20 ° C and that of the olive oil that varies: from 1.4670 to 1.4710 at 20 ° C [44], So we see that our oil is qualitatively clearer.

3. 3. Synthesis of biodiesel
3. 3. 1. Transesterification reaction yield

Biodiesel yield calculation is obtained by applying the following equation:

$$\eta = \frac{experimental\ mass\ of\ bio-diesel}{theorical\ masse\ of\ bio-diesel} \quad (3)$$

Practically the yield is : η = 92.56 %

The yield of the transesterification is very dependent on the operating conditions. In effect, too high a temperature of the reaction medium causes the degradation of the oil used

3. 3. 2. Obtained biodiesel Characterization
3. 3. 2. 1. Physical and chemical characteristics

The same parameters measured for the oil above, are determined also for the bio-diesel of this oil, in addition, a characterization by spectrophotometry IR is carried out too. The values of the measured parameters are summarized in the following table :

Table. 4. Characteristics of obtained bio-diesel.

Parameter	Measured value
Cinematic viscosityy *(ν)*	4.1647 Cp
Density (d^{20})	0.8554
pH	4.1
Refractive index	1.4575

The high viscosity of the vegetable oils, compared to that of diesel derived from oil, is the main reason for which these raw materials are converted into biodiesel, the high viscosity of the oils as fuel, creates operational problems in the engines. The petrodiesels often possess the values of kinematic viscosity in the range of 2 to 3 $mm^2\ s^{-1}$. While, the

range of kinematic viscosity in the standards of biodiesel goes beyond this range, as well as it is noticed that, most of biodiesel fuels present a kinematic viscosity varies between 4 and 5 $mm^2\ s^{-1}$ at 40 ° C; Note that there seems be no technical reason for which the kinematic viscosity is not the same for the Biodiesel and Petrodiesel. However, the structure of the compounds also impact significantly on the viscosity, this last, increases proportionally with the length of the chain; the kinematic viscosity is also strongly temperature dependent, it increases at low temperatures [13].
As regard to the density, our biodiesel to a 0.8554 density at 20°C, it is very near to that standard biodisel, whose density varies between 0.86 and 0.9; it is better by comparison with that given by Miao and Wu [45] for the biodisels emerged from the oleaginous plants which is 0.838.

As regards the pH, according to Muhammad Saad Khan and all [46], the pH of a diesel fuel is generally varies in the range of 3.6 to 5.6. In our case, it was obtained a pH of 4.1 which is in this range.
The refractive index is considered as being a property important for the characterization of the fuel; it is particularly employed for the calculations intermediary as content on hydrogen of hydrocarbons and also intercepts refractivity [46, 47, 48]. This parameter can be used as a reliable physical property to predict the progress of the reaction of transesterification [49]. The value of the refractive index of the diesel fuel often varies between 1.45 to 1.475 [46] in this study, the synthesized biodiesel having a value of the refractive index is 1.4575, it included well in these indicated values, it is also close in the values of diesel fuel oil and to biodiesel of which Refractive indexes at 25oC are 1.4650 and 1.4548 respectively.

*3. 3. 2. 2. Characterization by IR spectrophotometry*The IR spectrum (Fig. 4) of bio-diesel synthesized is carried out by the means of a IR spectrophotometry, type : Cary 600 series ATR FTIR Spectrometer, Brand: Agilent Technologies.

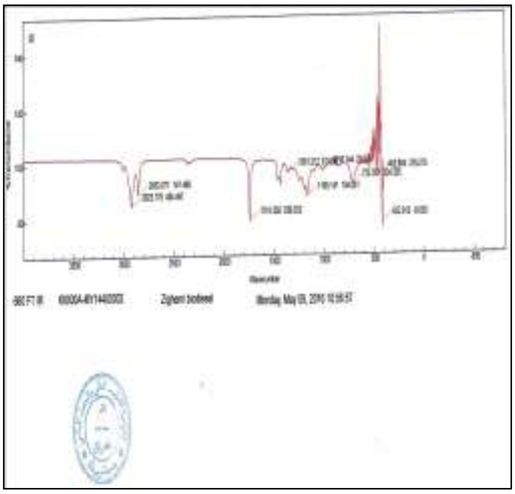

Fig. 4: IR spectrum of synthesized biodiesel.

According the IR spectrum (Fig.4.), the essential functional groups found confirms that the product is a biodiesel (ester) and are :

- An intense absorption at 1000 to 1300 cm^{-1} of the C-O ester, in our case this absorption at 1169 cm^{-1}.
- A band of elongation with High Intensity around 1750 to 1730 cm^{-1} of the connection C=O, in our case it is exactly to 1741 cm^{-1} of carbonyl group.
- Two bands of elongation with average intensity below 3000 cm^{-1} of C-H bonds saturated, for our biodiesel these bands are at 2922 and 2853 cm^{-1}.

3. 4. Comparison of characteristics of biodiesel and Chlorella algae oil

The comparison of some characteristics of our biodisel with those of *Chlorella* alga oil obtained as well as those of standard biodisel according the nome ASTM 14214, is given in the following table :

Table. 5. Comparison of characteristics of oil and biodiesel obtained.

Parameter	**Obtained oil**	**Obtained biodiesel**	**Standard biodiesel ASTM**
Viscosit*y (v) in Cp*	32.3394	4.1647	3,5 – 5,0 at 40 °C
Density *(d^{20})*	0.8714	0.8554	0,86 – 0,9 at 15 °C

The analysis of the results of table 5, we show that, our biodiesel is in conformity with the ASTM standard 14214 and it is preferable to the use as biofuel.

The standard EN 14214 is considered as being the most restrictive standard for the biodiesel of microseaweeds [50], indeed, the analysis of the results of table 5, we show that, our biodiesel is in accordance with the standard ASTM 14214 and it's better to use him as biofuel with regard to the oil extracted from the seaweed *Chlorella pyrenoidosa*.
Our biodiesel is characterized by a kinematic viscosity of 4.1647 Cp, which in compliance with the results of Johnson and Wen [51] which declared that the kinematic viscosity of biodiesel stemming from microseaweeds is included in the intervalley from 3.87 to 5.2 mm^2/s.
As regards the density, our biodiesel is characterized by a density of 0.8554 measured at 20°C, it is near with that obtained by Miao and Wu [45], who obtained a density of 0.864 mesued at 15.5°C for the biodiesel of seaweed *Chlorella protothecoides*, it is lower than that obtained by Alptekin E, Canakci M who showed that the density of biodiesel is vary between 0.86 - 0.89 [52], on the other hand, our profits is in accordance with the standard ASTM 14214.

4. Conclusion

Biofuels appear as an essential energy source of transport indispensable. One of the interesting sources of biofuels algae, which are able to produce biodiesel and bioethanol which can be used in various types of engine.

The objective of this work consists to synthesize the biodiesel from oil of a green alga which is the alga *Chlorella pyrenoidosa* by the transesterification, we obtained an oil output of about 40.17%, this oil undergoes a reaction of alcolyse, using methanol, for the reaction and the NaOH as a catalyst; the obtained output is 92.56 %, this output depends on the conditions of work and certainly on percentage of triglicyrides in the oil. The characterization of our biodiesel was started thereafter, we used the infrared spectroscopy to identify the biodiesel obtain, thing which confirms us that the synthesized biodiesel is well the methyl ester of fatty acids

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