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Production of 3rd generation biofuel from green microalgae : *Oedogonium nodulosum*

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***Abstract*— The socio-economic and environmental situation strongly marked by the increase in oil production and global warming has led researchers towards an alternative path such as renewable energies. The purpose of this study was to synthesize biofuel from oil extracted from green microalgae by the transesterification process using ethanol as alcohol and potassium hydroxide (KOH) as homogeneous catalyst for the reaction. *Odogonium nodulosum* was the selected green microalgae for our Biodiesel production. This species exhibited high capacity of lipid (45.38% in dry weight) which could be of high potential as raw materials for biofuel production, with an overall efficiency of 80%. Some physic-chemical properties and characterization of the obtained products were also verified such relative density, refractive index, kinematic viscosity, cloud point, color and Biodiesel flash point measurements. The comparison of our results with the properties of Diesel and Gasoline showed us that our biodiesel has properties very similar to those of diesel but more ecological.**

***Keywords*— Green microalgae**, ***Odogonium nodulosum*, Lipids extraction, Transesterification, Biofuel**

I. INTRODUCTION

With the continued development of industry and transport, energy demand in our world is increasing day after day. Oil, or even all fossil fuels (coal, natural gas and oil), currently provide almost all of this energy. However, these resources are not renewable and their consumption at the current rate will lead to the disappearance of reserves in a few decades [1]. On a global scale, the solar energy received represents about 10,000 times humanity's annual energy consumption. It is clearly the most abundant source of renewable energy [2]. Photosynthesis is an excellent natural way to convert and store solar energy [3]. The world of aquatic photosynthetic microorganisms, including microalgae, represents a considerable and almost untapped potential for bioenergy production [4, 5]. Indeed, these microorganisms are of great importance, due to their ability to produce high-value and energy-interesting compounds such as: fats, hydrogen, carbohydrates and proteins [6, 7]. Their use makes it possible to consider the development of innovative biofuel production processes [8].

Biofuels are intended to replace petroleum-based fuel. They are a sustainable alternative to fossil fuels as they are renewable and less toxic to the environment. For the time being, these biofuels are of vegetables origin and come from agricultural crops. A major disadvantage is the debate between the use of land to produce food or biofuel [9]. As a result, many studies have been conducted to use an alternative energy source, many of which focus on algae.

Indeed, the biomass from algae is convertible via chemical or enzymatic transformations into biodiesel or bioethanol. Almost all biomass can be transformed into transport energy. The lipids are converted into biodiesel and the rest of the biomass can be used to produce bio-methanol. The production of biofuel from algae has many advantages that go beyond replacing oil as transport energy [10, 11].

In fact, the purpose of this study is to synthesize a biodiesel from green microalgae oils by transestérification using a basic homogenous catalyst.

The most effective basic catalysts used for the transesterification of triglycerides include NaOH, NaOMe, KOH and KOMe [12, 13]. In addition, the basic catalyst technique in a homogeneous medium in the presence of methanol (MeOH), is the most commonly used because it is the cheapest and can be carried out at low temperatures and pressures [14].

II. METHODS AND MATERIALS

Microalgae samples were collected from a freshwater channel covered with filamentous algae according to the CIPEL protocol [15]. The samples were grown at laboratory scale in an algal photobioreactor in batch mode. A dominant green algae species has been selected for the production of biofuel. It is *Oedogonium nodulosum* (Fig. 1). The most important physical (light, agitation, temperature) and chemical (various nutrients) factors necessary for its growth and the culture medium used were considered [16]. This microalgae was initially grown in batch mode in 1.5 liter photobioreactors under sterile and aseptic conditions, while considering that

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light and carbon dioxide have an important influence on the cell growth of *Oedogonium nodulosum*.

Fig. 1 *Oedogonium nodulosum* (x400 magnification)

A biomass-concentration monitoring was carried out in order to recover the optimal amount of our selected species under optimal growth conditions (temperature (22°C), light (120µmol.m^{-2}.s^{-1}), agitation (100 rpm), dissolved CO_2 level (13%), pH ≈ 6 to 8, and nutrients). The maximum biomass growth rate µmax is calculated as follows:

$$\mu_{max} = \frac{log_2 N_n - log_2 N_0}{t_n - t_0}$$

With :

μ_{max} : maximum biomass growth rate (mn^{-1}) ; log_2 : decimal logarithm ; N_0 : Initial concentration of dry algal mass ($mg.L^{-1}$) that corresponds to the initial time t_0 (mn); N_n : Highest Biomass Concentration ($mg.L^{-1}$) corresponding to time t_n (mn).

II.1. Physico-chemical analyses

II.1.1. Determination of dry matter

To determine dry matter (DM) content of wet algal "pastes", a standard MB35 moisture meter was used. This equipment works on the principle of thermogravimetry, which consists in measuring the mass variation of a sample according to the temperature, which is fixed to 110°C until a constant mass was obtained [17]. Once the stabilized weight has been reached, drying is completed and the result displayed indicates the percentage of moisture present in the matrix and the final weight of the sample. Dry matter calculation of the algal paste was carried out as follows:

$$DM\% = 100 - HM\%$$

Avec : DM%: percentage of dry matter ; HM%: pourcentage d'humidité donné par l'humidimètre

II.1.2. Lipid extraction

Lipid extraction from dry Oedogonium nodulosum algae was performed using the Soxhlet system (ISO 659-1988) [18]. This method consists in extracting oils contained in the algal cells below 70°C using for our case a combination of two organic solvents: n-hexane and chloroform (2: 1). The solvent, contained in the flask, is boiled, which then transfers it to the upper part of the Soxhlet (Fig. 2). There, it is condensed by a refrigerant at the top of the installation and accumulates around and inside the cartridge. When the solvent reaches the upper level of the siphon, the mixture is returned to the tank by pressure difference, where it is evaporated again. Several extraction cycles are thus carried out, the duration of the operation is fixed at 4 hours according to the standard [18]. It is then considered to have reached total solute depletion of the solid substrate and concentrated the extract.

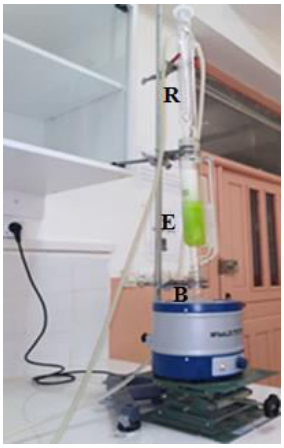

Fig. 2 Soxhlet diagram (source: Engineering Tech.) R : Refrigerant, E : Extractor, B : Balloon

The extracted oil was then placed in the Rotavapor flask to remove organic solvents in excess (Fig. 3) by distillation process at 70°C with 100 rpm [19].

Fig. 3 Rotavapor type R3000-Gemini BV for the distillation of extracted oil

II.2.1. Homogeneous transesterification reaction

A basic homogeneous transesterification reaction was conducted to transform the extracted oil into an ester [20]. 200 ml intake of oil extracted from Oedogonium nodulosum is placed in a tricols flask, to which 341 ml of methanol (MeOH) and 1.85 g of KOH were added. The proportion of the catalyst amount to be used varies within a range of 0.5 to 1% of the oil mass, for a conversion efficiency of 94 to 99% [14, 20]. The mixture is heated up rapidly to 70°C, for the reaction under mechanical agitation of 100 rpm [21]. The standard transesterification reaction is as follows:

$$Triglyceride + Alcohol \leftrightarrow Ester\ alkylique + Glycerol$$

However, the molar ratio alcohol/oil 9/1 chosen for our case of methanolic transesterification of the extracted oil, favors a better separation of glycerin because it increases its

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solubility [22]. Methanol is the alcohol used in our transesterification reaction, due to its better reactivity than ethanol [19, 22]. This efficiency advantage is that the use of methanol does not pose any difficulties during separation [20]. The water content of the extracted oil is 0.5 (% by weight), and the total phosphorus content was 0.8 ppm.

After 120 minutes of stirring, the resulting mixture is poured into a separating funnel and left to stand for 24 hours, in order to promote the separation of the biofuel from Glycerol/methanol.

The transition from an oil state to an alkyl ester state, commonly known as biodiesel, makes it possible to: reduce the molecular weight to one-third of that of the oil, reduce the viscosity by a factor of 8, reduce the density and increase its volatility [24, 25].

A purification step of the ester consists in removing impurities such as residual glycerin, excess alcohol, traces of catalysts, soaps and salts formed by the homogeneous catalysis. This purification operation is carried out by flash distillation under vacuum.

After washing the biodiesel with water, the drying operation allows the water present to be removed by heating the product to a high drying temperature (120°C) in the oven.

III. Results & Discussion

A. *Biomass Growth*

Based on microscopic examination, we mainly observed the abundance of chlorophyceae, and the culture conditions in the photobioreactor in batch mode showed a concentration of algal biomass of the *Oedogonium nodulosum* species, with the higher growth rate (μ_{max} = 4.9 days^{-1}) (Fig. 4).

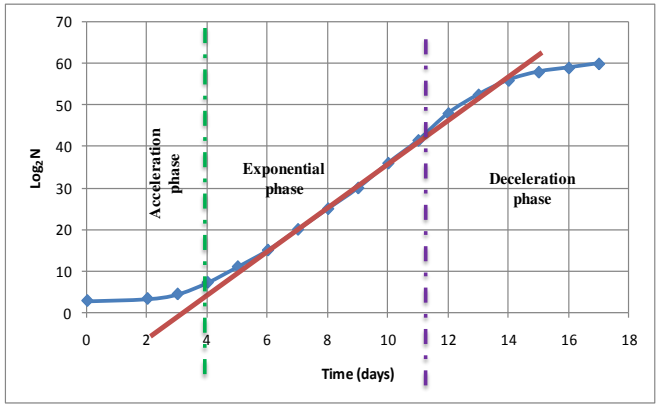

Fig. 4 : Biomass growth curve of the selected species *Oedogonium nodulosum*

From the maximum growth rate obtained, we can deduce that the maximum number N of Oedogonium nodulosum was reached from the 11th day of pure algal culture.

$$Log_2 N_n = X = 41.5 \qquad \text{where :} \quad N = 2^X = 2^{41.5} \approx 10^{18} \text{ cells.L}^{-1}.$$

B. *Characterization of the Extracted Oil*

This cultured algae paste was used for lipid extraction. After drying, we obtained a dry mass of *Oedogonium nodulosum* equal to 259.87g from a wet paste of 1.55kg. The mass of the extracted oil from this dry algal mass is 112.58g with an extraction efficiency rate of 43.32%.

C. *Transesterification Reaction Efficiency*

The influence of various transesterification reaction times (1, 2 hours) at temperature (70°C) was studied (mass ratio MeOH / oil of 9 /1 and 1% by mass KOH). The evolution of biodiesel yield (g biodiesel /g oil) over the time of reaction is shown in (Fig. 5).

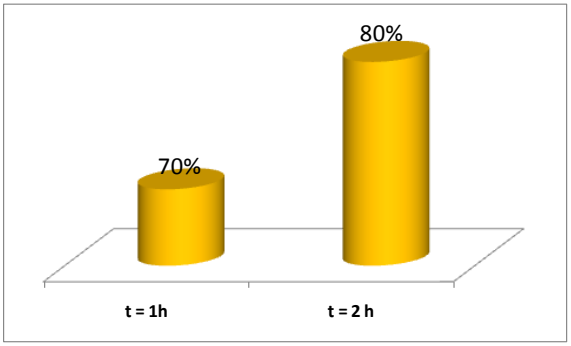

Fig. 5 Evolution of biodiesel yield according to reaction time (mass ratio MeOH/oil: 9/1 KOH : 1% mass, 70°C)

The transesterification reaction results show that the maximum biodiesel yield of 80% is obtained after 2 h reaction is complete. An efficiency of 70% is obtained after 1 hour of reaction at room temperature. Therefore, the reaction time seems to have an influence on biodiesel yield.

D. *Physicochemical Properties of Biodiesel*

The physico-chemical properties of biodiesel produced from *Oedogonium nodulosum* oil, compared to those of diesel fuel, are presented in Tab. 1.

Tab. 1 Physicochemical properties of biodiesel from *Oedogonium nodulosum* oil compared to diesel oil

Compounds	pH	Density g.cm^{-3}	Viscosity at 40°C mm^2.s^{-1}	Flash point °C	Cloud point °C
Oedogonium nodulosum oil	8.59	0,9236	0,663	155	-10
Biofuel	9.77	0,8484	1,8984	138	-12
Diesel Fuel	9.39	0,8393	2,8620	8	-14

Biodiesel has a lower density, viscosity and flash point than extracted oil. These lower physico-chemical properties of biodiesel compared to that of the oil correspond to those of methyl esters by glycerol removal. However, the density and flash point of biodiesel are higher than those of diesel. These results show that the combustion of the latter, particularly at start-up, is easier than that of biodiesel.

E. *Optimization of Biodiesel Yield*

In order to determine the optimal biodiesel yield, a series of transeterification reactions of the extracted oil were carried out under the following conditions: reaction time: 1h, temperature 70°C, mass ratio Methylene / oil: 12/1, 9/1, 6/1 with a mass percentage KOH / oil: 1%.

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Tab. 2 Biodiesel production results according to the alcohol/oil mass ratio

	1st trial	2nd trial	3rd trial
Alcohol / oil mass ratio	12 / 1	9 / 1	6 / 1
Yield (%)	79	80	68

The best separating result was obtained with a ratio of 9/1 (Fig. 6).

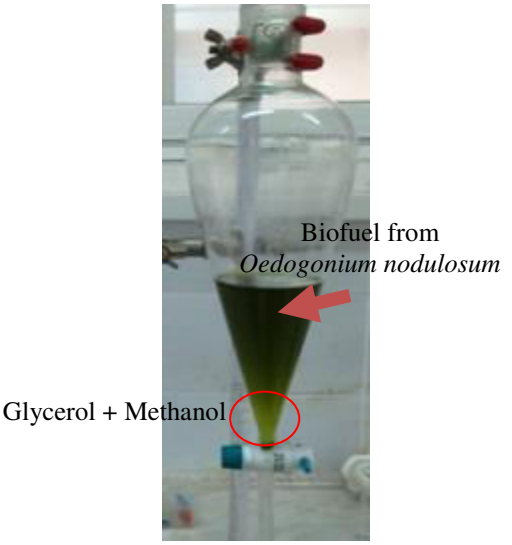

Fig. 6 Biodiesel produced by transesterification separated from the organic phase Glycerol/methanol

Glycerol is essentially insoluble in biodiesel, so almost all glycerol is easily removed by settling. Alcohol can act as a co-solvent to increase the solubility of glycerol in biodisel. In our case, the yield obtained is less than 100 (Tab. 2). This can be explained by the fact that glycerol has not been solubilized in biodiesel due to the transformation of glycerol into fatty acid which in turn has undergone saponification in the presence of KOH [26, 27, 28]. Indeed, during transesterification, parallel parasitic reactions occur at the same time [28, 29, 30]. Fatty acids react with a basic catalyst to form soap [31, 32]. This will inevitably reduce the conversion efficiency [33].

IV. Conclusion

From the standpoint of renewable energy and sustainable development, third-generation biofuels are attracting increasing investment interest from researchers and some green industries. Biofuels are emerging as an essential transport energy source. One of the interesting sources of biofuels are algae, which are capable of producing biodiesel and bioethanol that can be used in different types of engines. Biodiesel, which is derived from triglycerides methyl or ethyl alcohol by transesterification, has attracted considerable attention over the past three decades due to its benefits: renewable, biodegradable and non-toxic fuel. The objective of this work was to synthesize a biodiesel from green filamentous microalgae "*Oedogoium nodulosum*" oils, by transesterification, which is the most widely used technique for biodiesel synthesization. For this purpose, we used a short-chain alcohol (methanol) for the reaction and KOH as a catalyst. Parameters affecting alkyl ester yield such as catalyst mass percentage (1% KOH) and catalyst/oil mass ratio were studied over time.

The highest biodiesel yield obtained is 80% and corresponds to a mass ratio of 9/1 Methanol / oil, at a temperature of 70°C and a reaction time of 2 hours.

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