# Biodiesel from microalgae in lagoons: an acceptable alternative fuel to the transportation crunch?

**By**

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**ABSTRACT**

One of the major reasons for microalgae popularity as an acceptable fuel source is their potential to maximize the productivity, e.g. gallons of oil or fuel produced per acre per year, converting the energy of solar photons during the process of $CO_2$ fixation into biomass energy benefiting the environment. Marketing the generated co-products, termed post extraction algal residue (PEAR) could make the transportation fuel viable from microalgae. These self-contained microbial factories can convert sunlight and $CO_2$ into biofuel are the fastest growing plants and 50% of their weight is oil.

The main objective of this research is the qualitative and quantitative analysis of the biofuel obtained from microalgae and focuses on the energetic and environmental feasibility of this fuel for use in the transportation sector. This evaluation includes the identification of the obstacles and limitations of the process in terms of energy and environmental performance. Another important aspect of this work is the need for improvement of product processing and recovery that includes mechanical and energy intensive processes like centrifugation, filtration, flocculation, and cell disruption.

The results obtained so far show that the various strains of microalgae used (nearby lagoons in Portugal and Spain) offer a huge prospect of biodiesel production.

KEYWORDS: Biodiesel, microalgae, alternative fuel, lagoon.

## 1. MOTIVATION

The current oil crisis and the fast depletion of conventional fossil oil reserves have made it more imperative for organizations and countries to invest more time and efforts in research on suitable renewable feedstock. It is known that algae grown in $CO_2$ enriched environment can be converted to oily substances and hence can contribute to the solution of two major problems:

a) Reduction of gaseous emissions (fossil $CO_2$, nitrogen and sulphur oxides), namely greenhouse gases (GHGs).

b) Shortage of energy sources without compromising food production capacity.

The increasing fuel demand worldwide, rapid rise of crude oil price, limited reserves and the effects of fossil fuel use on the environment has pushed scientists to look for cleaner, renewable fuels. Biodiesel fuel has received considerable attention with its biodegradable, renewable and non-toxic characteristics. However, the production of biodiesel from cultures such as sun flower or rapeseed has several problems, namely: competition with food cultures, risk of distortion of food prices, effective reductions in terms of GHGs emissions, and need of environmental sustainability criteria for biofuels production. The main rationale underlying biodiesel utilisation emerges from the need to find an alternative energy source for the transportation sector.

## 2. THE ENERBIOALGAE PROJECT

The EnerBioAlgae Project is being developed during 2011-2012 as a part of the SUDOE Program (Territorial Cooperation Operational Program of Southwest Europe 2007-2013). The main goal is to study the potential for biodiesel production from microalgal species and its overall Life Cycle Analysis (LCA). The project partnership consists of three entities in Spain (University of Vigo as Coordinator, University of Almería and Inega – Energy Institute of Galicia), two in France (University of Pau and Pays de l'Adour and the Centre National de la Recherche Scientifique) and one in Portugal (University of Aveiro).

The secondary objectives of the project are:

- To refine the system of production of algal biomass for energy purposes in order to improve yields;
- To develop the methodological aspects and enhance the technical, economical, environmental and energetic potential of microalgae;
- To identify, locate and use the degraded water resources for high potential energy sources in Galicia (Spain), Almería (Spain) and Aveiro (Portugal);
- To develop instrumentation technologies for online monitoring and control of algae cultures with a system based on the use of LIDAR technology and pattern recognition algorithms that reveal the state of the algae at real time;
- To optimize the quality of biodiesel produced from microalgae (fulfilling the European regulation) to encourage public and private investment;
- To execute a complete economic analysis of the microalgae-derived biodiesel production;
- To evaluate and demonstrate the technical, economical and energy feasibility of the developed environmental technologies and to develop an overall well to wheel (WTW) LCA for the evaluation of the environmental impacts of the production of biodiesel from microalgae.

This research can be divided into five main tasks: a) allocation of resources, identification and characterization of the strain; b) selection and growth of suitable microalgae under proper conditions; c) online monitoring of the algae; d) extraction procedures; and e) analysis and characterization of the obtained oil.

The project integrates the protection and conservation of the environment, combating climate change, diversification of energy supplies, development and exploration of alternative energy and biodiversity. The use of microalgae as an energy source for conversion into biofuels has excellent prospects, with potential competitive advantages. The aim is to achieve maximum profitability of the energy production on an industrial scale, which requires more investment in research applied to various fields of technology and supported by pilot experiments for demonstration purposes. The EnerBioAlgae project addresses these needs while contributing to the improvement of the environment by integrating the use of contaminated water and $CO_2$ capture. Furthermore, microalgae cultivation does not compete with food production, does not require large area or fertile terrains and maximizes water savings. Microalgae cultivation offers significant environmental benefits when compared with crops such as rape, sunflower, jatropha etc. However, biodiesel from microalgae is still more expensive than conventional diesel. A recent estimate states that the cost of microalgae-derived biodiesel is on a 20 fold range over conventional diesel oil *(1)*.

The enhancement of the energy potential from a given territory and the mitigation of environmental issues on degraded water resources derived from EnerBioAlgae will stimulate the growth of the renewable energy sector in Southwestern Europe, contributing to energy diversification, reducing the dependence on fossil fuels and ensuring a quality supply of energy under the European standards.

## 3. LITERATURE REVIEW

Several authors addressed the production of biofuel from microalgae as a promising source addressing the issues of the current oil crisis and the greenhouse and global warming problems.

US DOE launched the National Algal Biofuels Technology Roadmap (*2*) that lays the groundwork for identifying challenges that will likely need to be surmounted for algae to be used in the production of economically viable, environmentally sound biofuels.

11.6 million tonnes of fuel were consumed in Portugal in 2011 (*3*). To satisfy that need by resorting to microalgae with an average lipid content of 30% requires only 3200 $km^2$ land while if sunflower cultures were used an area of about 4 times the area of Portugal would be required *(4)*.

In order to satisfy demand it is important to choose the most suitable microalgae (in terms of lipid content and growth rate) for biodiesel production, both from the points of view of quantity and quality of the oil. Physical (light and temperature), chemical (nutrients and $CO_2$) and biological factors (virus infections or contaminations) affect the growth of algae. The factors can be handled externally in order to produce high oil content biomass for biodiesel. Figure 1 lists the oil content of various microalgae and the factors influencing the growth rate of the microalgae.

**Factors that influence algal growth in a high rate algal pond**

| | |
|---|---|
| Abiotic factors | Light (quality, quantity) |
| Physical and chemical | Temparature |
| | Nutrient Concentration |
| | $O_2$, $CO_2$ |
| | pH |
| | Salinity |
| | Toxic chemicals |
| Biotic factors | Pathogen (bacteria, fungi, viruses) |
| | Predation of zooplankton |
| | Competition between species |
| Operational factors | Mixing |
| | Dilution rate |
| | Depth |
| | Addition of bicarbonate |
| | Harvesting frequency |

A)

| Microalga | Oil Content (% dry wt) |
|---|---|
| *Botryococcus braunii* | 25-75 |
| *Chlorella sp.* | 28-32 |
| *Crypthecodinium cohnii* | 20 |
| *Cylindrotheca sp.* | 16-37 |
| *Dunaliella primolecta* | 23 |
| *Isochrysis sp.* | 25-33 |
| *Monallanthus salina* | > 20 |
| *Nannochloris sp.* | 20-35 |
| *Nannochloropsis sp.* | 31-68 |
| *Neochloris oleoabundans* | 35-54 |
| *Nitzschia sp.* | 45-47 |
| *Phaeodactylum tricornutum* | 20-30 |
| *Schizochytrium sp.* | 50-77 |
| *Tetraselmis sueica* | 15-23 |

B)

**FIGURE 1 A) oil content of various microalgae strains *(5);* B) the growth rate of algae influenced by physical, chemical and biological factors *(6)***

Algae basic needs include water, sunlight and $CO_2$. Nutrients such as nitrogen (N), phosphorous (P) and potassium (K) can serve as fertilizer for the algae growth. Silica, iron and several trace elements can be considered as important marine nutrients. Potential algae biodiesel manufacturers can build biodiesel plants close to the energy manufacturing plants that produce lots of carbon-dioxide. The basic reaction in water is ($CO_2$ + light energy + $H_2O$ = glucose + $O_2$ + $H_2O$).

Some authors developed a study in which sea water was supplemented with commercial nitrate and phosphate fertilizers and a few other micronutrients for growing marine microalgae *(5)*. Sawayama et al. used some of the $CO_2$ that is released in power plants by burning fossil fuels for the growth of the microalgae *(7)*. Other authors found that beside carbon, nitrogen is the second most important nutrient to microalgae growth since it may comprise more than 10% of the biomass. Nitrogen can be present in many forms, but the most common nitrogen compounds assimilated by microalgae are ammonium ($NH_4^+$) and nitrate ($NO_3^-$) *(5, 8)*.

The microalgae cultivation is followed by extraction and esterification. Extraction methods are used to convert the chemical energy contained in the grown microalgae into high-value oils with minimal fossil energy consumption if possible. Triglycerides or triacylglycerol's have a glycerin backbone joined by ester linkages to three fatty acid chains. The fatty acid portion varies in length between twelve and eighteen carbons. Biodiesel molecules are mixture of fatty acids methyl esters (FAMEs) produced from a reaction named transesterification.

There are several physical (Mechanical crushing, Osmotic shock, Ultrasonic extraction) and chemical (Hexane, Soxhlet, Enzymatic, Supercritical fluids) extraction processes for extracting lipids from dry or wet algal biomass. Some authors mentioned that the combination of sonoenzymatic treatment accelerates extraction and increases the yield *(9)*. Other authors also address that the supercritical alternative is suitable for the biodiesel production from a technical point of view; however, from an economic analysis, it does not appear to be good *(10-13)*. Some other procedures were proposed by Bligh and Dyer *(14)*, who used solvent mixtures made of chloroform or methanol and ethanol or diethyl ether. Combining methods like microwave heating and Soxhlet extraction, reduced the extraction time from 8 hours to 32 minutes *(15)*.

The oil extraction process through the wet route consumes 2.8 times more energy than dry route (*16*). Fossil energy ratio (FER) of the dry route is slightly higher than that of the wet route. High FER of the dry route benefits from the high thermal efficiency (95%) of the pyrolysis process. Figure 2 shows the detailed dry and wet routes from microalgae production to biofuel.

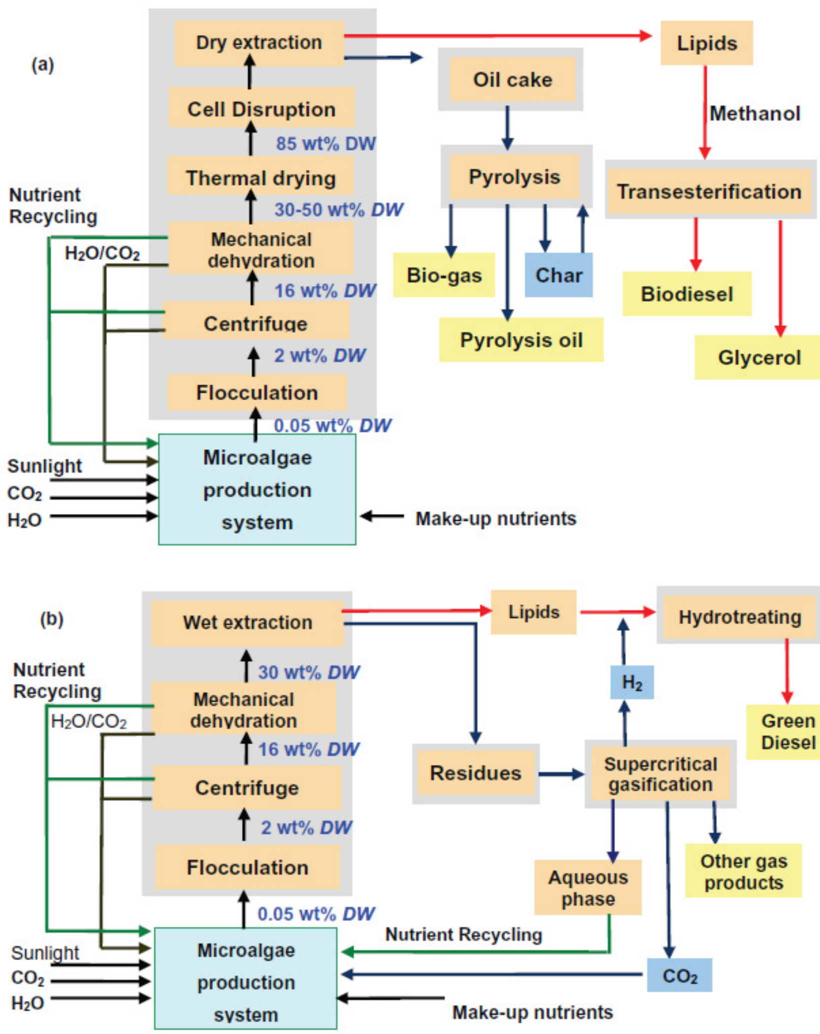


**FIGURE 2 Microalgae-to-biofuel route, (a) the dry route; (b) the wet route *(16)***

## 4. METHODOLOGY

In our work we have investigated the potential of the biodiesel produced from different microalgae strains like Chlorella vulgaris, Scenedesmus, Chlorophyta and Nannochloropsis grown in laboratory conditions. Early research on Chlorella vulgaris acknowledges that the lipid content by weight of dry mass under normal development is about 14%, while in freshwater by nutrient deprivation is 40% by weight of dry mass *(17)*.

A Pugh matrix (also known as Criteria Based Matrix) will be developed, in order to create a decision support tool that studies different criteria, for comparison of the trade-offs between them and also will highlight how the phases of the biodiesel from microalgae production can be optimized. Development of a Pugh Matrix plays a crucial role in the present proposal since this methodology will help to determine which potential solutions are better (based on criteria). It will be used for the selection between several viable choices with different options in order to assign scores relative to the criteria. More reliable results will be obtained if scores assigned are based on reliable data from the process. The expected results include a technical and economical characterization of the microalgae processing phases and biodiesel production, an economic model of the processing phase and a Life cycle assessment of the biodiesel production from microalgae. The developed LCA will appear as a decision-making tool. Since different levels of lipid production of microalgae will be obtained and knowing that biodiesel should specify quality regulations, the economic analysis appear to be the best chain of processes in its broadest sense, i.e. integrating LCA.

### 4.1. Experimental procedure

A photobioreactor (PBR), consisting of a stainless steel structure, 6 acrylic columns of 110 mm outer diameter and 10 fluorescent tube lights TLD 36W/865, each column with an approximate volume of 5 L, was used for the microalgae culture. There were two pH and temperature sensors placed in the middle of two columns. An initial inoculum of half liter per column of microalgae strain was used. Nutrients were added on an everyday basis.

For the cultivation the pH was maintained between 7.2 - 7.5, the culture temperature was around 22$^{o}$C and a photo-period of light/dark for 12 hours was used. Everyday 80ml of samples were collected from each columns and the same amount was restored by adding water and nutrients in order to calculate the cell growth on a daily basis. The maximum growth of cells was reached on the 9$^{th}$ day of culture. Figure 3 shows the PBR used for the cultivation of algae in our study and the culturing steps.

**FIGURE 3 Culture in column PBR installed in the University of Aveiro and extraction of samples**

Several strains were tested in the PBR, namely Chlorella, Chlorophyta and Scenedesmus. In addition, lyophilized biomass of Nannochloropsis and Scenedesmus were sent from Almería region to the University of Aveiro, for oil extraction.

Then, algal biomass was gathered, centrifuged and eventually transformed into a paste-like wet substance. Both wet and dry extraction were conducted with the cultured algae biomass. Lyophilized biomass was used for dry extraction process. The extraction process included magnetic stirring, vacuum filtration and ultrasound. The extracted oil after trans-esterification was characterized by GC. In our work the wet extraction procedure as a modification of method by Bligh and Dyer (*14*) was used to extract the lipid in micro algal cells. Extraction and partitioning of the lipids are synchronal in the Bligh and Dyer method *(14)* following an equilibrium theory. Addition of methanol can be one of the key parameters for the improvement of the lipid content following the extraction kinetics. Hence, tissues homogenized with chloroform-methanol are possibly the best lipid extractant despite the environmental and health hazards are likely to accompany it *(19)*.

## 4.2. Quality analysis

The crucial point for the production of high quality biodiesel is the fidelity to biodiesel fuel standard specifications, EN 14214 (2003). Within EN 14214, method EN 14103 specifies the Fatty Acid Methyl Ester (FAME) and Linolenic Acid Methyl Ester (LAME) content, which is used to profile the vegetable or animal oil feedstock used in biodiesel production. The FAME analysis was carried out with a split injection into an analytical column with polar stationary phase and an FID detector.

The equipment used here is the Varian 3800 Gas Chromatograph (GC), fitted with a capillary injector and FID. The inlet temperature was $220^{o}$ C, the injection volume was 2 µL, the oven final temperature was $200^{o}$ C and the column configuration is DB1-ht, 15 m, (0.32 mm x 0.1 µl).

An analysis based on the FAME, composition of saturated and unsaturated methyl esters, LAME and iodine value predicts the critical parameters and the overall potential of the biodiesel. It was concluded that the quality of biodiesel strongly depends on the growing process that should be optimized for the mass production. The technologies employed to purify and transform the feedstock into fatty acid alkyl esters determined whether the oil produced meets the specification standards but also conditions of the produced quantity of free and bonded glycerin which in return defines the purity and quality of the biodiesel.

FAME is determined by:

$$C = \frac{\sum A - A_{IS}}{A_{IS}} \times \frac{C_{IS} \times V_{IS}}{m} \times 100\% \qquad \textbf{Eq. 1}$$

Where:

$\sum A =$ Total peak area $C_{14:0} - C_{24:1}$;

$A_{IS}$ = internal standard (methyl heptadecanoate) peak area;

$C_{IS}$ = concentration of the internal standard solution, in mg/mL;

$V_{IS}$ = volume of the internal standard solution used, in mL;

m = mass of the sample, in mg.

According to EN14103, the result for the total FAME content should be higher than 90% *(20)*. Also, the method of oil extraction can directly influence the fatty acid profile of the lipid content of microalgae depending on the efficiency of extraction of polar and neutral lipids *(21)*.

LAME is calculated by:

$$L = \frac{A_L}{\sum A - A_{IS}} \times 100\% \qquad \textbf{Eq. 2}$$

Where:

$\sum A$ = total peak area $C_{14:0} - C_{24:1}$;

$A_{IS}$ = internal standard (methyl heptadecanoate) peak area;

$A_L$ = linolenic acid methyl ester peak area.

Total linolenic acid ($C_{18:3}$) content should be higher than 1 % and lower than 15 % *(21, 22)*.

The mass of iodine in grams absorbed by 100 grams of a chemical substance isby definition a measure of total unsaturation compounds in the fatty acids. The EN14214 specification allows a maximum of 120 for the iodine number (*23*).

Iodine value = X g iodine / 100 g sample **Eq. 3**

## 4.3. Life Cycle Assessment

A LCA broadens the scope from environmental impacts to three dimensions of sustainability (people, planet and prosperity) *(24)*. For this task the GREET model (developed by the Argonne National Laboratory) (*25*) was updated and adapted (since it contemplates the U.S. reality) in order to carry out the LCA of biodiesel from microalgae for the Portuguese reality. For the WTW analysis, a case study based on the 300kms route between Lisbon and Porto, using a passenger vehicle, was considered.

## 5. RESULTS AND DISCUSSION

### 5.1. Chlorella vulgaris from Aveiro region, Portugal

Figure 4 shows the fatty acid methyl ester (FAME) profile of wet extraction of biodiesel from Chlorella vulgaris while Table 1 shows the LAME and iodine value in accordance to the European standard. Total FAME is approximately 95 %.

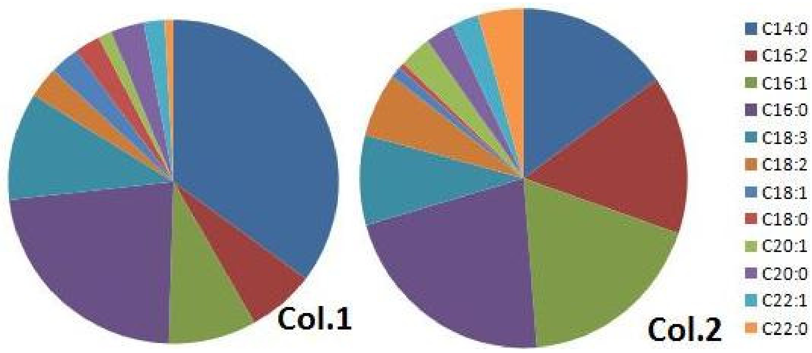


**FIGURE 4 FAME profile of wet extraction of biodiesel from Chlorella vulgaris**

**TABLE 1 LAME and iodine value of Chlorella vulgaris**

| Samples | LAME (%) | Iodine Value (%) |
|---|---|---|
| Column 1 | 13 | 13 |
| Column 2 | 9.4 | 9.4 |

Results confirm that Chlorella vulgaris grown with the lagoon water of Aveiro region in Portugal with an appropriate methodology and correct execution offers good potential to produce high quality biodiesel in accordance to the European standard.

### 5.2. Chlorophyta and Scenedesmus from Aveiro region, Portugal

Chlorophyta and Scenedesmus were cultured in parallel in the PBR using the water collected nearby the lagoon of Aveiro, in Portugal. Half of the biomass samples were kept for lyophilization (freeze-drying) in order to perform the dry extraction process. The remaining half was used for the wet extraction procedure.

Figure 5 shows FAME profiles with comparison between the wet and the dry extraction process, respectively for Chlorophyta and Scenedesmus. FAME analysis with wet extraction steps confirms that both the algae types are having high and almost equal potential in the production of biodiesel.

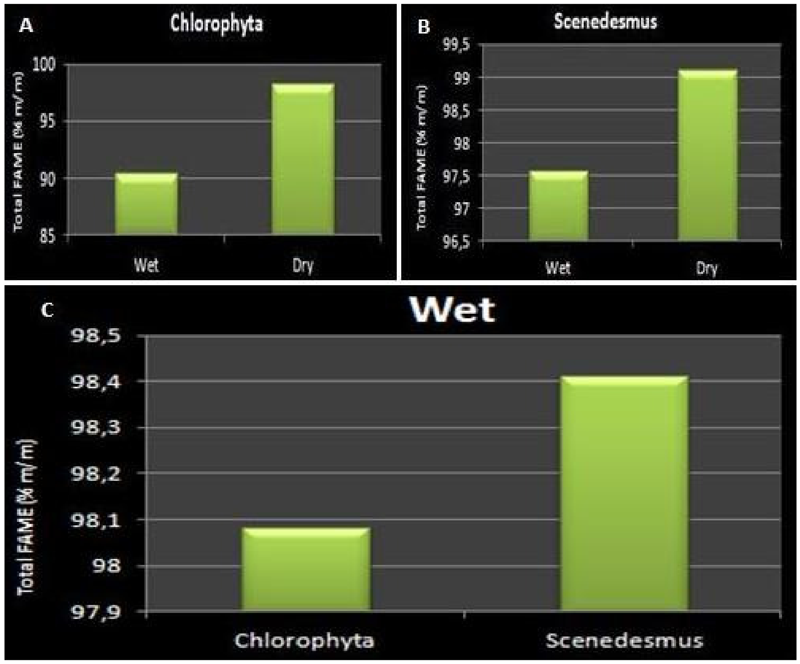


**FIGURE 5 FAME - A) Chlorophyta, B) Scenedesmus: Comparison of extraction processes, C) potential of the microalgae strains**

Thus FAME analysis confirms that the dry extraction techniques are more effective in order to produce high quality biodiesel irrespective of the microalgae type. Also, both the algae strains have equally high potential to produce quality biodiesel for the transportation sector.

LAME and Iodine values confirm both the strains to be have an high potential according to the EU standard, as presented in Table 2.

**TABLE 2 LAME and iodine values from Chlorophyta and Scenedesmus**

| Samples | LAME (%) | | Iodine Value (%) | |
|---|---|---|---|---|
| | Wet (%) | Dry (%) | Wet (%) | Dry (%) |
| Chlorophyta | 5.59 | 5.3 | 48.445 | 48.827 |
| Scenedesmus | 2.38 | 2.015 | 34.14 | 51.58 |

An integrated triangular graph (Figure 6) was plotted representing all the compounds (saturated, monounsaturated and polyunsaturated) in order to compare different parameters in respect to the European Standard EN 14214. The explanation of the colors is as follows: yellow (right), good cetane number and iodine value; yellow (left), good Cold filter plugging point (CFPP); green (intersection), biodiesel that satisfies UNE-EN 14214; orange, interest in biodiesel production is zero. The results obtained are in the area that meets the limit of the Cetane number and Iodine value (yellow area, right), which show a high content of saturated methyl esters according to the European standard EN14214. The most principal versed topic related to the iodine value of biodiesel is the oxidation stability since the oxidation process affects the fuel quality *(23)*. Thus, both microalgae Chlorophyta and Scenedesmus offer high potential for biodiesel production with an appropriate methodology and proper execution. Dry extraction process produces better results compared to the wet extraction process.

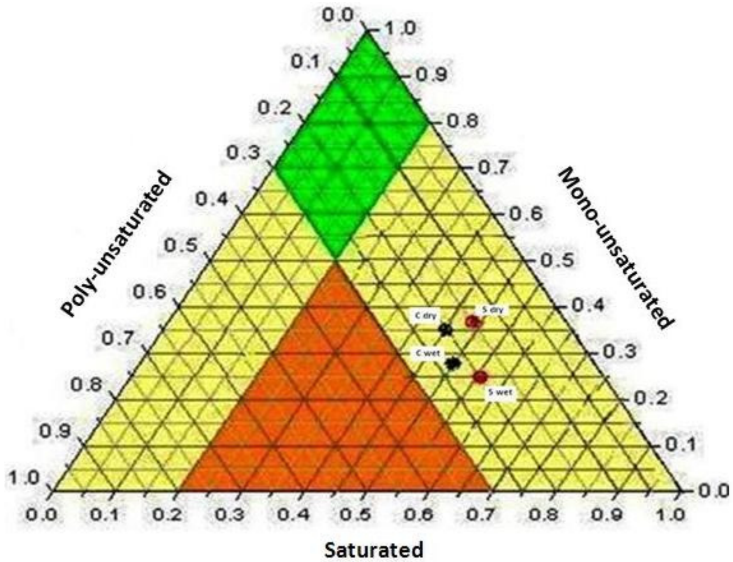


**FIGURE 6 Distribution of monounsaturated, polyunsaturated and saturated methyl esters – Chlorophyta and Scenedesmus (Aveiro, Portugal)**

## 5.3. Nannochloropsis and Scenedesmus from Almería region, Spain

Table 3 shows the total FAME and LAME values of the microalgae grown with local resources of Almería, Spain.

**TABLE 3 Potential strain in accordance to the EU standard**

| Samples | Total FAME % | Total LAME % |
|---|---|---|
| 1. Nannochloropsis [N] | 99.56 | 10.23 |
| 2. Scenedesmus [S(1)] | 100 | 4.5 |
| 3. Scenedesmus [S(2)] | 99.96 | 7.46 |

Figure 7 shows the good biodiesel production capability potential of all the strains. All the strains are in the green region of the graph, which means a very high and striking potential of the biodiesel production, both in terms of quality and quantity.

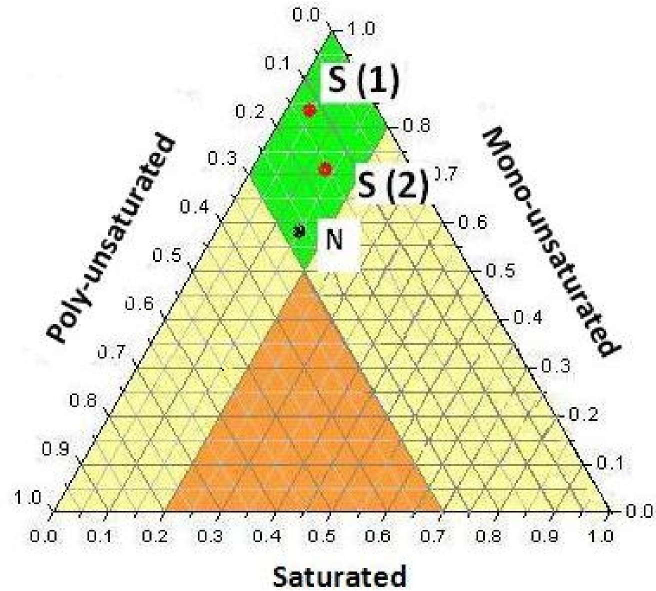


**FIGURE 7 Distribution of monounsaturated, polyunsaturated and saturated methyl esters – Nannochloropsis and Scenedesmus (Almería, Spain)**

## 5.4. Life Cycle Assessment

The preliminary results from LCA using the GREET model (updating it with data from the Portuguese reality, such as the electricity production mix, types of trucks and their European emissions regulations, types of trains, distances from the sea transportation sector) lead to the conclusion that biodiesel from microalgae is presented as a valid option to replace conventional fuels. For a WTP analysis, the reduction of GHGs emissions can go from 176% to 629% when compared with diesel. These values are obtained taking into account that the CO2 source is located in the same place of the biodiesel production. The transport of CO2 by heavy duty truck, when necessary, makes the biodiesel production process energetically and environmentally unviable, since energy consumption and GHGs emissions are at least 100% higher when compared to conventional fuels.

In a WTW analysis, the use of BD30 (30% of biodiesel mixed with 70% of diesel) presents a 42% lower fuel consumption than diesel; with BD50 and BD100, for BD50 and BD100 mixture the consumption increases between 20% and 40% respectively, relatively to BD30. When there is the need of transporting CO2, the energy consumption values are between 165% and 425% higher than a diesel vehicle. Regarding GHGs emissions, values are 82% lower than diesel for the BD100, presenting the emission of 33g of GHGs per km. In the stages of raw material and biofuel production there is a capture of 119 grams of GHGs from the atmosphere, making these stages environmentally positive.

It must be emphasized that while growing microalgae CO2 is used in diluted form. Even under the laboratorial conditions used in the PBR, CO2 was diluted in air. Also, pilot installations for microalgae cultivation do not attempt to separate CO2 from other combustion products. During LCA no attempt was made to address the issue of CO2 extraction and separation. The effects have not been considered because the main focus of our study was to produce high quality and quantity biodiesel from local algae strains and no attempts were considered concerning CO2 extraction and separation from other combustion gases. A LCA of the overall WTW is under development.

## 6. CONCLUSIONS AND FUTURE RESEARCH DIRECTIONS

This research has attempted to select microalgae from lagoons in order to produce an environmentally sound alternative fuel. $CO_2$ emissions have become a huge threat which can be solved by producing a fuel which uses $CO_2$ during its production. Microalgae can be a solution to solve this problem without compromising land resources for their production. Microalgae can be grown with degraded or wastewater and with $CO_2$ Intake. Choosing an inexpensive extraction process and reusing the co-products will help the feasibility of mass production of biodiesel.

Based on the values of the percentages of fatty acid methyl ester, saturated and unsaturated acids, linolenic acid methyl ester content and iodine factor, this research shows that the various strains of microalgae used offer a good prospect for biodiesel production. The production of the oil from dry algae biomass (dry extraction steps) gives better results than that obtained from the wet method, which contradicts some of previous work. The work was performed with the local strains of algae and algae biofuel extraction depends a lot on the particular strain and on the region of culture. The particular focus of the project was to come up with the potential biodiesel from local microalgae, both in terms of quality and quantity.

Further research will focus on the optimization of the algae growth conditions and the extraction steps (mechanisms leading to increased efficiency) enhancing the mass production of the biodiesel. Also, research with more varieties of microalgae strains is important in order to find the most viable and the best potential biodiesel source. Finally, LCA will be improved. It must be emphasized that the work conducted so far and described in this article does not assure commercialization at this stage, namely because the extraction and transterification procedures, although adequate from a laboratorial point of view, lack scalability.

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