Theoretical Estimation of the Production Potential of Biodiesel from Microalgae at the Talara Refinery

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Abstract- This study aims to calculate the theoretical potential that Talara region in Peru has for biodiesel's production from microalgae, capturing future emissions from the new Talara refinery which is currently in the modernization process (it will produce 2.5 million tons of CO_2 per year). The state of the art in the production of biodiesel from microalgae was investigated, reviewing the research carried out worldwide about biodiesel's production from microalgae, taking as a reference existing plants in Almeria (Spain) and using a mixed culture system: photobioreactor and open pond. The microalgae proposed to be used in the extraction of biodiesel is called *Dunaliella tertiolecta*. The theoretical plant of this study for biodiesel's production would allow to obtain 160 thousand tons per year, capturing approximately 300 tons per hour of CO_2 and considering only 30% lipids in the *Dunaliella tertiolecta* microalgae.

Keywords Microalgae, *Dunaliella tertiolecta*, photobioreactor, open pond, biodiesel.

1. Introduction

Modern industrialized societies are faced with a complex global energy landscape, which faces the problem of depletion of natural resources and climate change. The main axis of reduction of the increase in average temperature on a global scale is the gradual decarbonization of economic sectors that use large volumes of fossil resources, which are the main pollutants of the environment and contribute significantly to global warming.

In the World Energy Outlook 2012, an annual periodical of the International Energy Agency (IEA), the prediction of demand for liquid fuels in the year 2035 would reach 100 million barrels per day [1]. In this projection to the year 2035, it is expected that the production of crude oil from the current fields in operation will decrease and the production of Natural Gas Liquids (NGLs) will increase slightly. In other words, the International Energy Agency assumes that conventional crude oil production will be a thing of the past. [1].

In 2014, the U.S. Energy Information Administration published in its record called International Energy Outlook 2014 a prediction of the production of unconventional liquid fuels to the year 2040. According to the aforementioned prediction, unconventional liquid biofuels would reach a production of 3 million barrels per day by 2040 [2].

As specified by the International Energy Agency, in 2018, the global outcome of produced liquid biofuels reached 154 billion liters per year. In relation to 2017, there was an increase of 10 billion liters. Likewise, a 25% increase in liquid biofuel production is expected by 2024, approximately 40 billion liters [3]. The countries that will contribute significantly to the increase in liquid biofuel production projected for the year 2040 will be the United States, Brazil and China.

On the other hand, the International Renewable Energy Agency, IRENA, considers that, in order to meet the commitments of the Paris Agreement, by 2050 the production of biofuels must quadruple, compared to 2018, and reach an annual production of approximately 650 billion liters per year [4].

Currently Germany, France and Spain concentrate the majority of biomass production plants from microalgae in Europe. The most used systems in biomass production plants from microalgae are photobioreactors, representing 71% of the total, while open pond systems and fermenters represent 19% and 10% of the total production units, respectively [5]. The importance of using microalgae as an alternative to produce biofuels, is fundamentally because it reduces the use of biomass from corn and soybeans, avoiding the reduction of food for people (land use change), it reduces pollution by the use of chemicals and pesticides for the production of transgenics, capture CO_2 from industrial processes [6] [7].

In different regions of the world, policies have been developed to promote biofuels, with the aim of increasing their share of total global demand for liquid fuels [8] [9] [10]. Likewise, they want to diversify their energy matrix with renewable energies [11] [12] [13].

In Peru, in the last two decades there has been a worrying divergence between the demand and production of oil. As domestic demand for petroleum products increases rapidly, domestic oil production is inexorably reduced.

The dependence on oil in the transport sector is the weakest link in the Peruvian economy. According to the report "National Energy Balance 2014", 85% of the consumption of petroleum products in Peru is destined to land, air and sea transport, of people and goods. Therefore, it is essential to propose the general guidelines of a long-term strategy of depetrolization of transport in Peru.

According to information from Perupetro, in 2019, the maximum crude oil production in Peru was reached in November 2019, with a total of 63,700 barrels per day, which coincided with the maximum demand for liquid fuels, which exceeded 284,000 barrels per day, Fig. 1. Domestic production barely satisfies 32.8% of the total demand for liquid fuels, i.e., more than 67% comes from imports [14].

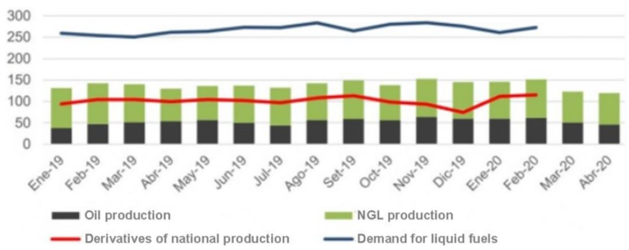

Fig. 1. Evolution of the demand and production of liquid hydrocarbons between January 2019 and February 2020 in thousands of barrels per day [14]

Prior to the spread of Covid-19 in Peru, the crude oil trade balance already reflected symptoms of contraction. This one improved due to a greater reduction in local oil imports (7.7 MBPD) by 7% compared to the 12% drop in Peruvian exports (0.8 MBPD) [15].

Peru is currently largely an importer of fossil fuels to ensure the sustainability and growth of the national economy. Thus, a progressive change in the national energy matrix is necessary, opting for alternatives such as biomass-based biofuels.

In 2018 the import of biodiesel was as follows: 3,552.0 TJ of ethanol and 7,561.8 TJ of biodiesel [16].

In September 2021, the demand for liquid fuels in the country was 209.78 MBPD, equivalent to 1,162.9 TJ. DB5 S-50 (a blend of Diesel No. 2 with S-50) constitutes 62.85% of the total. [17] [18].

It should be taken into account that, in sales to the Peruvian domestic market, gasoline is sold mixed with 7.8% of ethanol, called Gasohol, and Diesel 2 mixed with 5% biodiesel B100, called Diesel B5. In the National Energy Plan 2014–2025, they only contemplate solving the technical problems associated with oil of palm for the biodiesel's production, but do not consider the possibility of integrating the production of biodiesel from microalgae into the domestic market. The implementation of energy audits should be considered to determine the reduction in energy consumption and the impact on the environment, calculate CO_2 emissions and financial savings that would be obtained with current technology, and project targets in order to comply with the Kyoto agreement. The energy audit will serve to evaluate the feasibility of the biodiesel blends to be used as fuel [19]. A biofuel performance certification method is necessary in the biofuel industry to authenticate biodiesel and its blends for use in diesel engines. Brake Specific Energy Consumption (BSEC) is one of the key parameters for calculating the correct energy consumption of biodiesel and diesel blends [20].

The energy and fuel matrix in Peru must become progressively independent of the import of fossil fuels. This will allow the impacts on price variations and availability of fuels, not to be affected by events such as pandemics or others.

The production of biofuels from vegetable oils such as corn and soybean – ethanol and biodiesel respectively – has negative environmental effects due to the use of agrochemicals [6]. Likewise, the change of land affects the production of vegetables for human consumption, also implying a negative effect on the livelihood of families with reduced purchasing power due to higher food prices.

The production of biofuels from microalgae is projected as the best alternative to be used in the future due to the high production rates of fatty acids and lipids compared to the vegetables currently used [21] [22] [23]. In several investigations, the feedstock represents more than 75% of the total cost of biodiesel production, so it is important to find a way to optimize this so that this alternative is competitive compared to fossil fuels [21].

The use of microalgae as feedstock for the production of biodiesel is a great alternative due to the following characteristics: high lipid content, suitable profile for obtaining biofuel, relatively high growth rate, high photosynthetic efficiency, ability to develop both in marine waters, sweet and residual [24]. Additionally, microalgae can grow by bioconversion of CO_2 from industrial emissions, that is, they can capture CO_2 and reduce environmental pollution [25] [26] [27].

Considering all generations of feedstock for biodiesel, microalgae seem to be the only source of biodiesel that can be developed in a sustainable way in the future, capable of covering the world's demand for fuels, mainly for the transport sector [21].

The productivity of biodiesel from microalgae is hugely higher than the one from vegetables. Microalgae with medium oil content reach biodiesel productivities of 86,515 kg ha^{-1} yr^{-1}, microalgae with high oil content present productivities of 121,104 kg ha^{-1} yr^{-1}. Vegetables such as jatropha, sunflower and oil palm present productivities of 656.0, 946.0 and 4747.0 kg ha^{-1} yr^{-1} respectively [28]. We must mention that the studies carried out worldwide confirm that a biorefinery model for biofuel's production and value-added products based on microalgae makes it sustainable and profitable [29].

The technical specifications of biodiesel for the Peruvian Technical Standard 321.125.2008 have been taken from the American standard ASTM D6751. The energy content of biodiesel based on microalgae does not vary significantly with respect to fossil diesel, having a lower calorific value of 38,115.00 kJ kg^{-1} [30], equivalent to 16,420.93 BTU lb^{-1}.

The principal objective of this study is to investigate the theoretical potential for the production of biodiesel in the Talara Refinery (Peru), once the modernization project of this facility is completed, as well as to confirm that the Talara region meets the climatic conditions necessary for guarantee the optimal performance of any of the microalgae species used commercially for the production of biodiesel.

2. Methodology

2.1. Microalgae selection

The most studied species for biotechnological applications are green algae and diatoms [31].

The following figure shows the most commercialized microalgae species at present and their main ways of exploitation. Fig. 2.

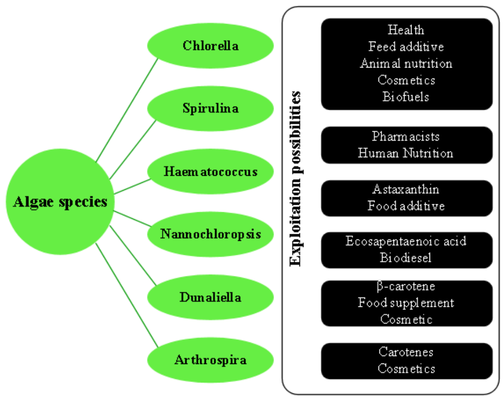

Fig. 2. Exploitation possibilities of the main commercialized microalgae species [32]

The following factors necessary for the culture of microalgae should be considered [33]:

- Environmental factors such as carbon dioxide, light, nutrients, temperature and salinity condition the photosynthesis and productivity of cellular biomass
- Ambient temperature is the second most important limiting factor for microalgae growth after light. The suitable temperature for growing microalgae is between 20 and 24 °C, however, this varies depending on the culture medium, the species and the strain of microalgae. At temperatures below 16 °C growth slows and temperatures above 35 °C are harmful to many species of microalgae [34].
- Nitrogen is a critical nutrient involved in lipid metabolism in microalgae. Microalgae require inorganic nitrogen and depending on the amount used many species can be induced to accumulate substantial amounts of lipids. The percentage of lipids varies between 1 and 70% but under certain conditions some species can reach 90% of dry weight [28].

On the other hand, microalgae can mitigate the effects of sewage effluents and industrial sources of nitrogenous waste, such as those originating from water treatment. Also, by removing nitrogen and carbon from the water, microalgae can help reduce eutrophication in the aquatic environment.

Microalgae with high lipid productivity are ideal for the production of biodiesel. The percentage of lipids contained in the biomass, the growth rate, the metabolic efficiency and the robustness of the species are very important parameters for the selection of microalgae [35] [36]. Species that bring out more than 30% fat materials are called 'oilseeds' [31]. Not all microalgae lipids are satisfactory for the production of biodiesel, however, those appropriate for this purpose such as fatty acids, free and covalently bound to glycerol, are frequently produced and represent the highest percentage of total lipids, between 20% and 40% [35]. A profile of long chain fatty acids with a low degree of unsaturation is recommended for the production of quality biofuel from microalgae [37]. The microalgae genera that have been investigated for the production of biodiesel are many, but the most reported are green microalgae, especially *Chlorella*, *Scenedesmus*, *Chlamydomonas*, *Nannochloris*, *Nannochloropsis*, and *Neochloris* [38]. **Error! Reference source not found.**. shows that both *Chlorella emersonii* and *Scenedesmus obliquus* have a high lipid content, however, volumetric and biomass productivity are very low. The microalgae that stands out for its high percentage of lipids and high volumetric and biomass productivity is *Dunaliella tertiolecta*.

Table 1. Lipid content and productivity of commercial microalgae for biodiesel production

Marine and freshwater microalgae species	Lipid content (% dry weight biomass)	Volumetric productivity of biomass (g L^{-1} d^{-1})	Areal productivity of biomass (g m^{-2} d^{-1})
Chlorella emersonii	25.0 – 63.0	0.036 – 0.041	0.91 – 0.97
Dunaliella tertiolecta	16.7 – 71.0	0.32	14
Nannochloropsis	12.0 – 53.0	0.17 – 1.43	1.9 – 5.3

sp.			
Scenedesmus obliquus	11.0 – 55.0	0.004 – 0.074	2.43 -3.5

Based on the information provided by the Solar Energy Research Institute, SERI, the most promising species are *Nannochloropsis salina* and *Dunaliella tertiolecta* due to their high concentration of fatty acids. Likewise, the National Renewable Energy Laboratory, NREL, in the United States, reported that *Dunaliella*, *Scenedesmus* and *Chlorella* are the most popular genera that have been successfully cultivated on a commercial scale to obtain biodiesel [39].

The microalgae selected for this research paper was *Dunaliella tertiolecta*.

2.2. Site selection

The biodiesel plant will be located in the vicinity of the new Talara refinery, currently in the modernization process, to take advantage of CO_2 emissions. The proximity to the coast will allow the use of seawater, which will allow the supply of the necessary nutrients in the microalgae culture stage.

The city of Talara is located in the region of Piura on the shores of the Pacific Ocean, with a warm, desert and oceanic climate. The average annual maximum and minimum temperature in the period 1950–2020 was 27.6 °C and 18.2 °C respectively, and with a total annual rainfall for the same period of 1,673.0 mm per month.

Dunaliella tertiolecta can easily adapt to a wide range of light intensity, from 50 to 2000 μmol photons m^{-2} s^{-1} [40]. This implies that *Dunaliella tertiolecta*, can grow smoothly in Talara region, as irradiances of 2350.1 μmol m^{-2} s^{-1} (equals to 22.56 MJ m^{-2} d^{-1}) are recorded [41].

The Piura region has an average solar irradiance value that ranges between 5.5–7.0 kWh m^{-2}. To determine the hours of light and therefore of daily operation of the culture stage, Matlab software was used. Matlab' calculations show that twelve hours of daylight are recorded in the Piura region, a parameter that is introduced into the biomass production calculations from microalgae.

The new Talara refinery, currently under construction, will have a hydrotreating and selective hydrogenation unit for naphthas, which implies high energy consumption and CO_2 emissions. Taking into account the modernization of the Talara refinery, CO_2 emissions will increase to 2.5 million tons yr^{-1}, some 6850 tons CO_2 d^{-1}, or 300.34 tons hr^{-1}. This last value indicates the maximum amount of CO2 that could be used to obtain biodiesel from microalgae, once the refinery modernization affair has been completed [42].

In general, for each ton of dry biomass, 1.83 tons CO_2 is needed.

The oil obtained from the processed biomass will depend on the percentage of lipid content of the cultured microalgae strain. There are strains of algae with oil content greater than 80% and less than 20%. The yield of the transesterification process to obtain biodiesel from the lipids of biomass from microalgae is of the order of 90%. That is, for each ton of oil we, 900 kg of biodiesel are obtained [42].

If the cultured microalgae strain had a lipid content of 30%, around 86.7 tons of microalgae oil would be produced per year in the Talara refinery for each mixed culture unit, consisting of a photobioreactor and an open pond. If the efficiency of the transesterification process was 99.5%, the biodiesel production would be 160,922.32 tons yr^{-1}, considering the capture of approximately 300.00 tons hr^{-1} of CO_2.

The next figure, Fig. 3., shows the theoretical location of the biodiesel production plant. The area that plant would occupy would be a total of 2465 Ha. The culture area would occupy approximately 1559 Ha, resulting from the calculations to obtain the yields of Table 4 and Table 5. As an initial biomass of 135 kg is considered in the culture with photobioreactors, to obtain the initial concentration of 337.5 g m^{-2}, an area of 400 m^2 is needed, comprising ten (10) photobioreactors of 3 m^3 each. In the case of the open pond, to obtain the concentration of 16.9 g m^{-2}, 8000 m^2 would be required. In total, each "photobioreactor + open pond" set would comprise 8400 m^2 (0.84 Ha) and since 1856 "photobioreactor + open pond" sets are required, the area that culture stage would occupy would be 1559.04 Ha.

The remaining 906 Ha correspond to the downstream process area. For the approximate calculation, the study carried out by the National Renewable Energy Laboratory (NREL) was taken into account, which in its technical report NREL/TP-5100-72716 [43], indicates that they use approximately 311.4 Ha to process 190,000.00 tons of biomass per year and as our plant in theory would process 552,744.18 tons of biomass per year, 906 Ha would be required for this purpose.

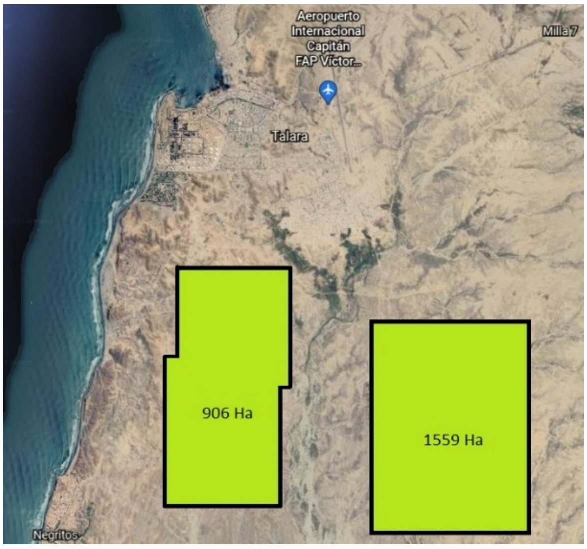

Fig. 3. Satellite view of the Talara Refinery (Source: Google Earth)

2.3. Description of the production phases

The following figure shows the main blocks of the biodiesel production process from microalgae, normally considered in the different studies carried out worldwide [28]. Fig. 4.

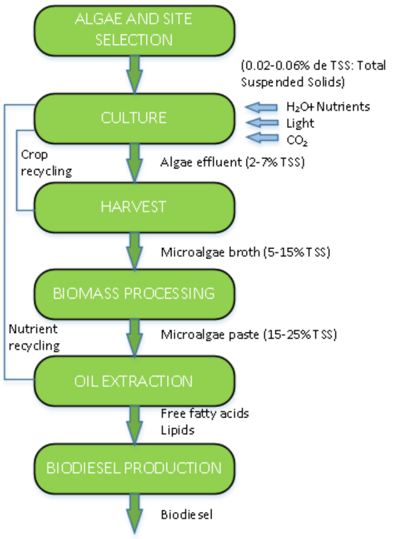

Fig. 4. Overview of biodiesel production processes from microalgae (Own elaboration)

Many studies take into account the following processes for the production of biodiesel from microalgae:

Culture

Three different metabolisms are considered for the production of microalgae, following the natural growth processes [34]:

- Photoautotrophic production
- Heterotrophic production
- Mixotrophic production

Photoautotrophic production uses light as the only source of energy, which is converted into chemical energy through photosynthesis reactions. The two culture systems most used in photoautotrophic production correspond to photobioreactor and open pond technologies.

Microalgae culture in open ponds has been used since 1950. These culture systems can be classified into [44]:

- Natural waters, such as seas, rivers, lakes and ponds
- Artificial ponds, road ponds, raceway ponds

The CO_2 requirements of microalgae are normally satisfied by surface air, but submerged aerators must be installed to improve CO_2 fixation [44].

Large–scale microalgal cultures usually take place in open cultures [38]. In an open pond culture system, it is difficult to avoid biological contamination by external organisms.

The water level cannot be below 15 cm or 150 L m^{-2} for photons of solar energy to reach the microalgae in the growth phase [45]. As the atmosphere only contains 0.03–0.06% CO_2, microalgae cell growth is limited.

The 'raceway' type ponds consist of circuits from 15 to 30 cm deep, inside them a paddle wheel maintains a constant flow of the culture, normally 0.5 m s^{-1}. Biomass production and productivity in these systems are low, close to 1 g L^{-1} and 10–25 g m^{-2} d^{-1} respectively. The inherent advantages of open crops lie in their simplicity and low investment cost compared to closed systems [24].

Open systems have several drawbacks such as [46] [47] [35] [48] [49] [50] [51]:

- Water losses due to evaporation
- Limited transfer of CO_2 to the culture due to its low concentration in the air, 0.035% of the dissolution volume
- Diffusion into the atmosphere
- Limited control of growing conditions
- High susceptibility to contamination, except for cultures of two extremophilic species
- Requirement of extensive surfaces
- Long production periods, between 6 to 8 weeks
- Reduced biomass productions and limited light penetration

Microalgae production based on closed photobioreactors overcomes several of the major problems inherent in open pond systems. There is less risk of biological contamination in photobioreactors, thus allowing the culture of a single species of microalgae for long periods of time [44].

The most widely used types of photobioreactors are the following [52]: flat plate, tubular, suspended bags, column and closed tanks.

Photobioreactors have many advantages over open systems. Depending on the design or shape, it is considered that:

- Grow conditions and parameters can be better controlled, including: pH, temperature, N, CO_2 and O_2
- They don't allow the evaporation of water
- They avoid CO_2 losses
- They achieve higher levels of biomass concentration

With the use of photobioreactors, the microalgae densities are higher, which represents a higher volumetric productivity. Likewise, they offer a safer operating environment, avoiding contamination or invasion by other microorganisms.

The mixed or hybrid culture, used in this study, is a method that combines two different growth stages, both in open ponds and in photobioreactors.

In general, such systems consist of an initial stage of biomass production in closed photobioreactors, in which microorganisms are kept in continuous growth under conditions of nutrient sufficiency. The next stage is a phase of accumulation of lipid production in open ponds, which is induced by nutrient deficiency [50].

The following parameters are mostly used to evaluate productivity in microalgae production plants:

- Volumetric productivity (VP): It is the productivity for each unit of volume and its units are given in g L^{-1} d^{-1}
- Areal Productivity (AP): It is the productivity obtained by each unit of occupied area and its units are given in g m^{-2} d^{-1}
- Illuminated Surface Productivity (ISP): It is the productivity obtained by each areal unit of illuminated surface and its units are given in g m^{-2} d^{-1}

Stress conditions, associated with nitrogen limitation, addition of metal ions and salts such as EDTA, increase lipid accumulation [53] [54].

Harvesting

Algae harvesting is the process of recovering biomass from the growing medium, which represents 20–30% of the total cost of biomass production [55]. To select the appropriate harvesting technique, the following will be taken into account: the type of microalgae, size, density and value of the target products, water content, salinity, robustness of the microalgae, deformation characteristics [38].

This stage highlights the difficulty of processing the tiny size of the microalgae, usually between 3–30 μm in diameter. The diluted state in which they are found, less than 0.5 kg of dry biomass per m^3, implies handling large volumes to obtain a significant amount of product [55].

Acceptable humidity is another consideration when selecting the harvesting method. The most widely used harvesting methods are: centrifugation, sedimentation, ultrafiltration, filtration, flocculation or a combination of flocculation-flotation.

The different existing harvesting techniques are conditioned by the following aspects:

- The characteristics of microalgae
- The cost of the technique to be used
- The final product you want to obtain

Chemical flocculation and centrifugation are the most economically viable harvesting techniques [38]. The sedimentation technique is based on the separation of suspended microalgal cells using gravity sedimentation.

According to Stoke's Law, it is established that the sedimentation rate is proportional to the radius of the cells and the difference in density between the microalgae and the medium. Therefore, each species will have a specific sedimentation rate. Conventional sedimentation has collection efficiencies ranging between 60%–70% [56] and its reliability is low due to the variability in size and density that different microalgae species can record. This technique usually requires long periods of time for effective separation, observing deposition velocities that usually range between 0.1–2.6 cm h^{-1}. These long deposition times could lead to the deterioration of the biomass during the process [57]. These factors make this harvesting technique only viable in microalgae that exceed sizes of 70 μm [58].

The application of the sedimentation or centrifugation technique could be feasible in microalgae with diameters greater than 5 μm and relatively thick cell walls.

The centrifugation technique is a reliable, fast and valid technique for a large part of the microalgae species studied. As in gravity sedimentation, it is based on Stokes' Law, in which the density and radius of microalgae conditions the sedimentation of suspended solids. The centrifugation technique uses centrifugal force for the separation of solids and liquids of different density, achieving the sedimentation of those particles of higher density and then separating the surplus, corresponding to the liquid phase. The efficiency of the centrifugation technique is conditioned by the particular characteristics of the microalgae species used., by the centrifugation speed, the residence time and the depth of the container in which the biomass is located [55]. In addition, it should be considered that the centrifugation technique can damage the cellular structure of microalgae, due to the centrifugation stresses to which they are subjected during the process [59].

Centrifugation, together with flocculation, represents one of the most efficient and best yielding techniques for the separation of biomass from microalgae culture [60]. Efficiencies of 95% can be achieved by increasing the concentration of the sludge up to 150 times [61]. However, it is inadvisable to use this technique to treat large volumes of crops because the cost and the time required would considerably increase the biomass collection process.

The centrifugation is only suitable for products with high added value, since it implies high costs and demands a high energy consumption.

Oil processing and extraction

The oil processing and extraction phase represents an important economic limitation for the production of low-cost basic products, such as fuels and food, as well as for higher-value products, such as β-carotene and polysaccharides.

To improve the usefull lifetime and the final product, harvested biomass dehydration techniques are typically used. After the drying process, the cellular disruption of the microalgae is carried out in order to release the necessary metabolites. There are several methods and the use of one or the other depends on the wall of the microalgae and the nature of the final product, being able to use mechanical methods such as cell homogenizers, ball mills, ultrasound, autoclave and spray drying; or using non-mechanical methods such as freezing, organic solvents with osmotic shock and acidic, basic and enzymatic reactions [4].

On the other hand, there are oil extraction methods, such as ultrasound and microwaves, which, compared to conventional methods, allow to reduce extraction times and increase yields by 50–500%, reducing costs and minimizing toxicity [28].

The lipid extraction process can be carried out in two different ways:

- From dried algae: the collected biomass is subjected to an extraction process in which it is previously dried. In this way, high percentages of lipid recovery are achieved, although it is associated with greater energy consumption, due to the drying operation. This alternative is not recommended for the treatment of large volumes of feedstock, although ways to make this process more efficient are being investigated [62]

➢ From wet algae: the collected biomass used in the extraction process contains a considerable percentage of moisture. In this case, the microalgae maybe subjected to a previous cell disruption, which allows to deteriorate or break the cell wall in such a way that the subsequent extraction of lipids is facilitated. Unlike the dry extraction process, wet extraction does not get percentages as high, however, the energy balance is much more favorable [63]

The most widely used lipid extraction methods are the use of pulsed electric fields, supercritical fluids, solvents, microwaves and ultrasound [64] [65].

Supercritical extraction consists of obtaining product by using CO_2 in a supercritical state. This technological process has advantages such as low extraction temperatures; no organic solvents are used, therefore there is no emission of volatile organic products. The yield of the oil extraction process is about 95% [66].

Wet extraction is an innovative method, developed by OriginOil Inc.; this method, called OriginOil Single-Step Extraction™, Fig. 5., performs the extraction directly from the aquatic culture environment, grouping the harvest, drying and extraction in a single step. To achieve the extraction, Quantum Fracturing™ would be used, a technology that combines the pulsation of electromagnetic fields with the modification of pH to break the cell walls, releasing the oil from the cells. This microalgae oil extraction process is more efficient and simple than the aforementioned systems, there is no need to use chemicals or invest in heavy machinery. Furthermore, the energy used to break down the microalgae cells is significantly less than other extraction methods, resulting in oil separation, water recycling, and biomass shedding. This innovative method allows to extract approximately 97% of the lipids contained in the cells of the algae, a value higher than the method of extraction by pressure (75%) and close to the method of extraction with supercritical fluid (100%). This process does not require heavy machinery, chemical reagents, or prior water separation. The whole process takes less than an hour.

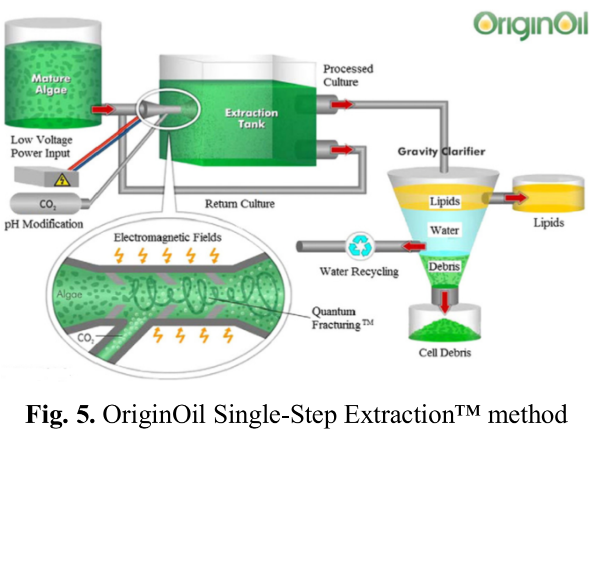

Fig. 5. OriginOil Single-Step Extraction™ method

Biodiesel production (transesterification)

Biodiesel is made up of fatty acid alkyl esters, produced by transesterification of animal fats, vegetable oils and microalgae oils. In this transesterification process, the transfer of an acyl group occurs, which can occur between an ester and an alcohol (alcoholysis), between an ester and an acid (acidolysis) or between esters (interesterification) [67].

These lipid feedstocks are mainly made up of 90–98% (weight) by triglycerides and then in smaller amounts by mono and diglycerides, free fatty acids (1–5%). Minimal amounts of carotenes, tocopherols, phospholipids, phosphatides, sulfur compounds and water are also present.

Transesterification is a multi-step reaction, including three steps in sequence. First, the triglycerides are converted to diglycerides; second, the diglycerides are transformed into monoglycerides and finally the monoglycerides are transformed into esters (biodiesel) and glycerol (by-product) [68].

To improve the speed of the transesterification reaction, a basic or acidic, heterogeneous or homogeneous catalyst is commonly used. For some processes that use supercritical fluids such as ethanol or methanol, it is probably not necessary to use this catalyst.

The most widely used catalyst is sodium hydroxide in a proportion of 0.5–1.0 g of catalyst for each gram of oil.

Alternatives to alkaline transesterification include: acid transesterification, microwave or ultrasound assisted, sub-critical or super-critical transesterification, heterogeneous transesterification, among others. These options are still under study.

2.4. Operating parameters of the biodiesel system from microalgae

Culture phase

The culture phase is made up of a mixed or hybrid system. This phase is related to the daily values of solar radiation, especially photosynthetically active radiation or PAR. PAR radiation can be defined as a fraction of the solar spectrum that is between 400 nm and 700 nm, in the visible area of the spectrum. This radiation is absorbed by plants, stored and transformed through their photosynthetic systems [69].

PAR radiation is the energy source for photosynthesis, which is generally estimated as a constant fraction of global solar radiation, frequently found at 35% to 50%, depending on weather and climatic conditions [69].

For microalgae, light is considered an indispensable substrate for growth. The Monod equation is used to model the growth of photosynthetic microorganisms, with the growth rate (μ) being dependent on the average irradiance (I_{av}). Monod's model results in equation (1) [70]:

$$\mu(I_{av}) = \frac{\mu_{máx} * I_{av}^{\ n}}{I_k^{\ n} + I_{av}^{\ n}} \qquad (1)$$

Where $\mu_{máx}$ is the maximum growth rate, I_{av} the average irradiance, I_k is the affinity to light and n is the shape parameter.

In the case of closed culture, the type of continuous operation is used, so the mass balance results in equation (2):

$$\mu - m = D \tag{2}$$

Where μ is defined as the specific growth rate, m is the specific maintenance rate, and D is the dilution rate.

Once the dilution rate (D) has been fixed, equation (3) is used to calculate the volumetric productivity based on the biomass concentration (C_b):

$$P_v = C_b * D \tag{3}$$

Where P_v is the volumetric productivity, C_b is the biomass concentration and D is the dilution rate.

For the culture in open ponds, a batch operation is used with the purpose that the biomass accumulates a greater amount of lipids due to nutrient limitation, the corresponding mass balance is obtained from equation (4):

$$\mu - m = \mu_{net} = \frac{1}{C_b} * \frac{dC_b}{dt} \tag{4}$$

Clearing the biomass concentration C_b, the final biomass concentrations are obtained with equation (5):

$$C_b = C_{b0} * e^{\mu_{máx} * t} \tag{5}$$

Where μ is the specific growth rate, m is the specific maintenance rate, C_{b0} is the initial biomass concentration, C_b is the final biomass concentration, and D is the dilution rate.

The culture phase has the following operating parameters:

- Ingress of algae : 0.02%
- PBR concentration : 0.45% (4.5 g L^{-1})
- Pond concentration : 0.07% (0.664 g L^{-1})
- Excess of CO_2 : 20.0%

Extraction phase

The wet extraction phase has the following operating parameters:

- Efficiency : 97.0%
- CO_2 input : 10.0% (kg CO_2/kg algae)

Transesterification phase

The transesterification phase has the following operating parameters:

- Efficiency : 99.5%
- Free fatty acids : 15.0%

Flash separation phase

The flash separation phase has the following operating parameters:

- Output of methanol : 4.0%
- Output of H2O : 2.0%
- Biodiesel efficiency : 1.0%
- Glycerin efficiency : 1.0%

Methanol separation phase

The methanol separation phase has the following operating parameters:

- Output of methanol : 100.0%

3. Results

The next figure, Fig. 6., shows the main blocks of the biodiesel production process considered in the present research. It should be indicated that the extraction process is carried out by direct wet extraction, for this reason a block of harvest as such is not shown, since it is unnecessary.

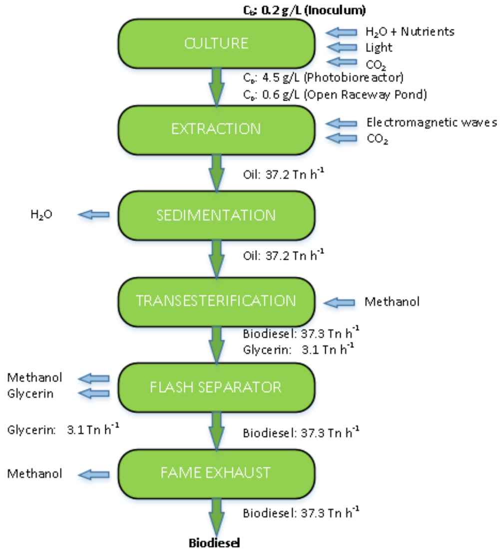

Fig. 6. Overview of the production processes of biodiesel from microalgae considered in this research (Own elaboration)

The mass balance formula for the production of biodiesel using microalgae presents different variants in terms of the coefficients for nutrients [71] [72]. In the present research the following formula is used for mass balance:

$$CO_2 + 0.915H_2O + 0.11N + 0.01P = CO_{0.48} + H_{1.83} + N_{0.11} + P_{0.01} + 1.2175O_2 \tag{6}$$

Equation (6) results in the nutrient consumption per kg of biomass, Table 2.:

Table 2. Nutrient requirement in kg per kg of biomass (Own elaboration)

Supplies	Quantity	Units
CO_2	1.88	kg
H_2O	0.70	kg
Nitrate (KNO_3)	0.48	kg
Phosphate (Na_3PO_4)	0.07	kg

O_2	1.67	kg

3.1. Culture phase

This phase is formed by a mixed or hybrid system. It is an efficient method of large-scale culture [73]. The first stage consists of photobioreactors and the second one of open ponds type raceway pond.

The objective of the first stage is to achieve the highest possible cell density, minimizing the risks of contamination and guaranteeing ideal conditions of nutrient sufficiency. In the second stage, lipid biosynthesis is stimulated, but under nutrient-limited conditions. Therefore, it is expected to obtain a high cell density and a high oil content.

The next figure, Fig. 7., shows the block diagram that interconnects the open pond with the photobioreactor. The photobioreactor block is made up of 10 vertical tubular serpentine photobioreactors [74]. The open pond block is formed by a (01) pond of 8000 m^2.

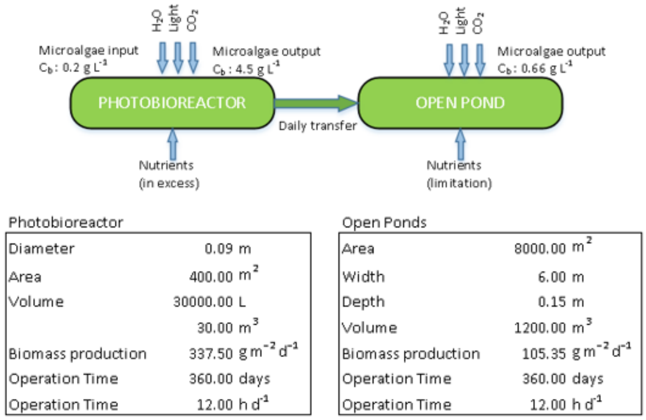

Fig. 7. Block diagram of the mixed or hybrid culture phase (Own elaboration)

Table 3. is a summary of the values of concentrations required in open ponds and photobioreactors, as well as the percentages of excess nutrients and CO_2.

Table 3. Summary of input and output concentrations required in the culture stages (Own elaboration)

Inputs		
Excess of CO_2	20.00	%
Excess of nutrients	2.00	%
Ingress of algae	0.02	%
Dilution air speed	0.50	%
Outputs		
Algae concentration in PBR	0.45	%
Algae concentration in pond	0.07	%

Since an excess of CO_2 of 20% is considered for the culture stage and 10% of CO_2 is used for the extraction stage, approximately 240,674.03 kg h^{-1} of CO_2 is available for the process, considering that for each kg of microalgal biomass 1,881 kg of CO_2 are used, approximately 127,950.04 kg h^{-1}, Fig. 10., it is obtained the outcome of the culture stage.

In this study, the values of volumetric productivity and areal productivity in Table 4. were considered, based on the design procedure proposed by Huntley [75].

Table 4. Design parameters for the culture phase (Own elaboration)

Culture system & data set	Initial concentration		Growth period (d)	Final concentration		Biomass per day (kg)
	g m^{-3}	g m^{-2}		g m^{-3}	g m^{-2}	
PBR	4500.0	337.5	n/a			135.0
Open pond	112.5	16.9	1	680.6	122.2	816.7

The theoretical annual production of biodiesel from this study is shown in Table 5.

Table 5. Theoretical annual production of biodiesel from microalgae (Own elaboration)

Culture system & data set	Oil production		Biomass production (g m^{-2} d^{-1})	Annual oil production (ton yr^{-1})	Annual oil production (ton yr^{-1}) - corrected
	%	g m^{-2} d^{-1}			
PBR			337.5	14.6	
Open pond	30	31.6	105.3	91.0	86.7

For the design of the photobioreactor and open pond, the operating values of the existing plant in Almeria, Spain, Fig. 8., were taken as a reference. The Almeria plant has an annual production of 4.58 tons yr^{-1} of dry biomass, considering the species *N. Gaditana*, with 21.8% lipids [75]. It should be noted that the climatic conditions of Almeria are very similar to those found in Talara, so it is a good reference for research.

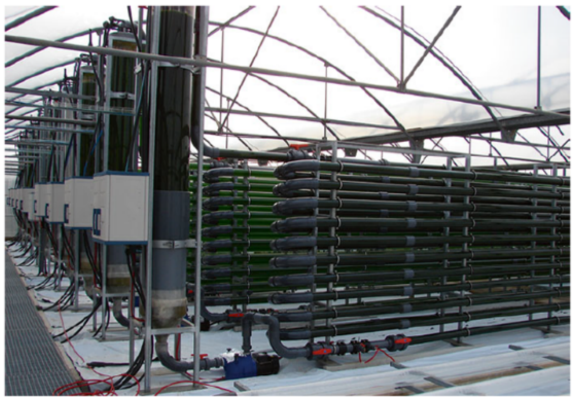

Fig. 8. Vertical tubular serpentine photobioreactor of Almería (Spain)

The photobioreactor of the present research covers an area of 400 m^2 and the open pond an area of 8000 m^2.

The biomass concentrations in the photobioreactors are between 4–6 g L^{-1} [73] [76]. In other researches consulted, concentrations between 10–16 g L^{-1} have been achieved using flat photobioreactors [77]. Microalgae biomass concentrations up to 10 g L^{-1} cans be achieved if photobioreactors are well designed [73].

The biomass concentration of microalgae in open ponds should be close to 0.65 g L^{-1} [76]. Microalgae biomass concentrations in open ponds between 0.5–1 g L^{-1} are accepted as standard [73].

The biomass concentrations considered in the present study are: in the photobioreactor it is 4.5 g L^{-1} and in the open pond it is 0.68 g L^{-1}. These values correspond to a single set "photobioreactor block + open pond block". To take advantage of the annual CO_2 production of the Talara Refinery, equivalent to 300,000.00 kg hr^{-1}, 1856 "photobioreactor block + open pond block" sets should be used. Each "photobioreactor block" consists of ten (10) 3000 L photobioreactors (40 m^2 area) and each "open pond block" consists of one (01) 1200 m^3 raceway pond (area of 8000 m^2).

The use of 1856 units "photobioreactor block + open pond block" would theoretically allow to obtain 160,922.32 tons of biodiesel per year, considering the use of the species *Dunaliella tertiolecta*, with a conservative percentage of 30% lipids, but with the probability of reaching 70% lipids, increasing the concentration of NaCl [78].

From the Hosseini's study it was discovered that increasing 0.5 or 1.0 mol L^{-1} of NaCl in the middle or at the end of the logarithmic phase of the *Dunaliella* species culture, considering at the beginning a NaCl concentration of 1.0 mol L^{-1}, raised the cell lipid content up to 70% [79].

Table 6. shows the results of growth tests of the microalgae *Dunaliella tertiolecta* with nitrogen limitation, obtaining up to 73% of unsaturated fatty acids [78].

Table 6. Fatty acid (FA) composition of algae oil extracted from algae for different nitrogen deficiency conditions

Composition FAME (%)	Biodiesel based on D. Tertiolecta	
	Normal growth	Nitrogen-limited growth
C16:0	28.1 ± 0.1	26.4 ± 0.06
C16:1	0.0	0.0
C16:2	2.8	2.3
C16:3	1.37 ± 0.06	1.27 ± 0.06
C18:0	0.6	0.6
C18:1	19.3 ± 0.15	16.8 ± 0.1
C18:2	14.67 ± 0.15	13.07 ± 0.23
C18:3	33.2 ± 0.26	39.6 ± 0.1
Sum of SFA	28.7 ± 0.1	27.0 ± 0.06
Sum of UFA	71.3 ± 0.1	73.0 ± 0.06

The mass balance for the photobioreactor is shown in Fig. 9., considering a continuous operation mode.

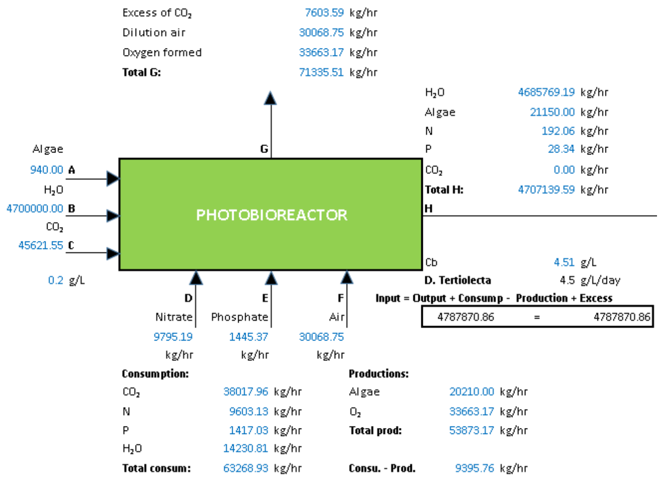

Fig. 9. Mass balance for the Vertical tubular serpentine type photobioreactor (Own elaboration)

Table 7. summarizes the mass inputs and outputs to the photobioreactor, based on nutrient consumption data obtained from the mass balance.

Table 7. Summary of mass inputs and output in photobioreactors (Own elaboration)

Inputs		
Algae (dry base)	940.00	kg h^{-1}
H_2O	4700000.00	kg h^{-1}
CO_2	45621.55	kg h^{-1}
Nitrates	9795.19	kg h^{-1}
Phosphates	1445.37	kg h^{-1}
Dilution air	30068.75	kg h^{-1}
Outputs		
Algae PBR	21150.00	kg h^{-1}

The mass balance for the open pond is shown in Fig. 10.

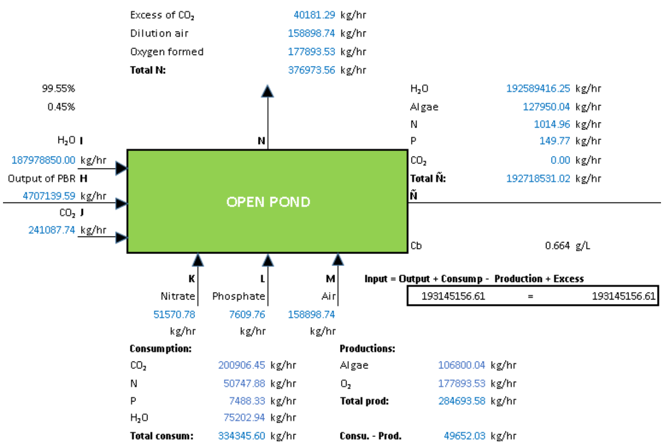

Fig. 10. Mass balance for the open raceway pond (Own elaboration)

Table 8. summarizes the mass inputs and outputs to the open pond, based on nutrient consumption data obtained from the mass balance.

Table 8. Summary of mass inputs and output in open ponds (Own elaboration)

Inputs		
H_2O	187978850.00	kg h^{-1}
CO_2	241087.74	kg h^{-1}
Nitrates	51570.78	kg h^{-1}
Phosphates	7609.76	kg h^{-1}
Outputs		
Algae open pond	127950.04	kg h^{-1}

The photobioreactors will operate in continuous mode and the open ponds will have a batch type operation.

3.2. Extraction phase

The present study considers the method of wet extraction of lipids from microalgae, which performs the direct extraction of the aquatic culture medium. It bundles both harvesting, drying and extraction in one step. It combines the pulsation of electromagnetic fields with the modification of pH to break the cell walls, releasing oil from the cells.

The extraction considers an efficiency of 97% and as mentioned above, a 30% lipid content in the species *Dunaliella tertiolecta*. The mass balance is shown in Fig. 11.

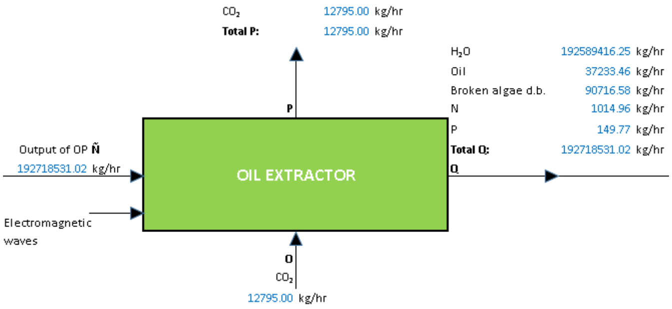

Fig. 11. Mass balance in the biodiesel extraction phase (Own elaboration)

Table 9. summarizes the inputs and outputs in the oil extractors. In this phase the algal biomass is transformed into oil.

Table 9. Summary of mass inputs and outputs in oil extractors (Own elaboration)

Inputs		
H_2O	192589416.25	kg h^{-1}
Algae	127950.04	kg h^{-1}
CO_2	12795.00	kg h^{-1}
Outputs		
H_2O	192589416.25	kg h^{-1}
Oil	37233.46	kg h^{-1}
Broken algae (dry base)	90716.58	kg h^{-1}
CO_2	12795.00	kg h^{-1}

3.3. Transesterification phase

The process of heterogeneous catalyzation is more efficient than the process of homogeneous catalyzation. The heterogeneous procedure, by catalytic transesterification of vegetable or animal oils or fats, with alcohols of low molecular weight, is basically carried out under conditions of mild temperature and atmospheric pressure.

The transesterification stage considers an efficiency of 99.5%. The mass balance is shown in Fig. 12.

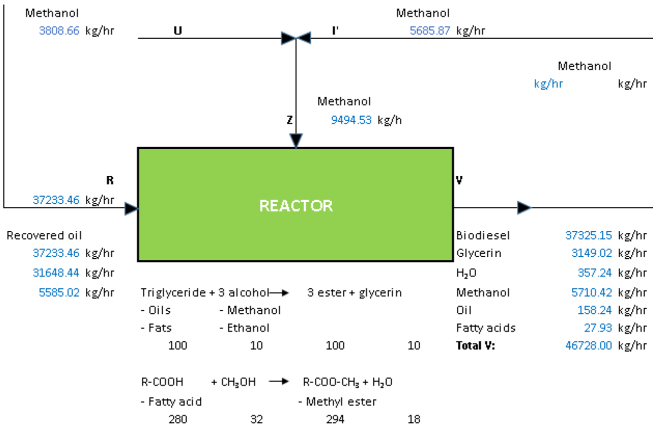

Fig. 12. Mass balance in the transesterification phase (Own elaboration)

The type of transesterification reactor to be used would be a fixed bed, which uses a patented catalytic process, ENSEL®, from the company Benefuel. This process combines the esterification of free fatty acids and triglyceride transesterification within the same step [80].

Table 10. summarizes the inputs and outputs in the transesterification reactors. In this phase the oil is transformed into biodiesel and glycerin as a by-product.

Table 10. Summary of inputs and outputs in transesterification reactors (Own elaboration)

Inputs		
Oil	37233.46	kg h^{-1}
Methanol	9494.53	kg h^{-1}
Outputs		
Biodiesel	37325.15	kg h^{-1}
Glycerin	3149.02	kg h^{-1}
H_2O	357.24	kg h^{-1}
Methanol	5710.42	kg h^{-1}

3.4. Separation phase of biodiesel and methanol–glycerin

A flash separator is used. Its main function is to separate the unreacted methanol from the biodiesel–glycerin mixture, in order to return it to the process, thus making the process or general production of biodiesel more efficient.

The mass balance is shown in Fig. 13.

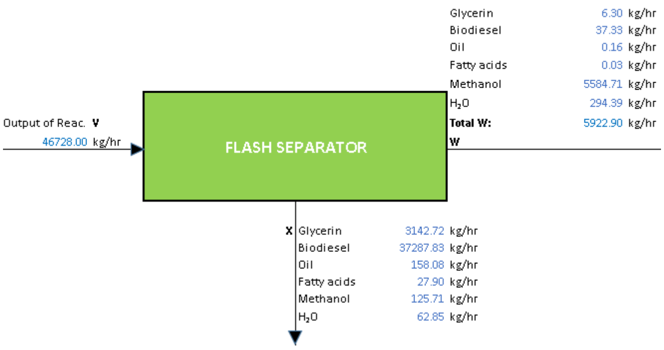

Fig. 13. Mass balance in the separation phase of biodiesel and methanol (Own elaboration)

Table 11. summarizes the mass inputs and outputs in flash separators. In this phase, the methanol is mainly separated, which is returned to the process.

Table 11. Summary of mass inputs and outputs in flash separators (Own elaboration)

Inputs		
Biodiesel	37325.15	kg h^{-1}
Glycerin	3149.02	kg h^{-1}
Methanol	5710.42	kg h^{-1}
Outputs		
Biodiesel	37287.83	kg h^{-1}
Glycerin	3142.72	kg h^{-1}
Methanol	125.71	kg h^{-1}

3.5. Separation phase of biodiesel and methanol

In the last stage of the downstream processes, 37,250.54 kg hr^{-1} of biodiesel are obtained and since the operating parameters that were taken as a reference indicate that the plant operates 12 hr d^{-1} for 360 days, the final result is that the biodiesel production reaches 160,922.32 tons yr^{-1}. The corresponding mass balance is shown in Fig. 14.

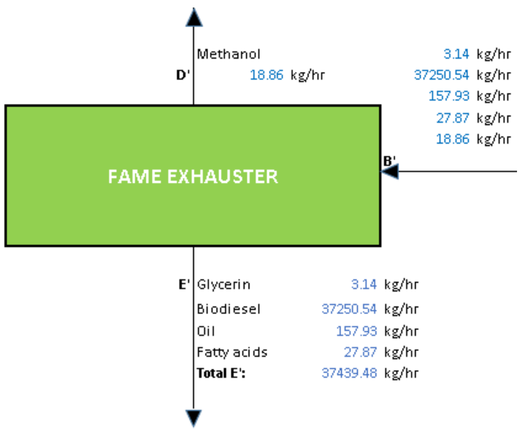

Fig. 14. Mass balance in the separation phase of biodiesel and glycerin (Own elaboration)

The exhausting FAME separates 100% of the methanol that enters this stage, and then returns it to the process.

Table 12. shows the summary of mass inputs and outputs in the exhausting FAME stage. In this phase, the methanol is separated to return it to the process.

Table 12. Summary of mass inputs and outputs in the exhausting FAME stage (Own elaboration)

Inputs		
Glycerin	3.14	kg h^{-1}
Biodiesel	37250.54	kg h^{-1}
Methanol	18.86	kg h^{-1}
Outputs		
Glycerin	3.14	kg h^{-1}
Biodiesel	37250.54	kg h^{-1}

4. Conclusions

The calculations made, taking as reference scientific studies consulted, the climatic conditions of the Talara region and the operating parameters of an existing plant in Almería (Spain), indicate that theoretically a biodiesel production of 160,922.32 tons yr^{-1}can be obtained. The above considering only 30% lipid content in the selected species, *Dunaliella tertiolecta*.

The irradiation conditions in the city of Talara are favorable so that the volumetric and areal productivities allow the mentioned production. The temperature variations in the City of Talara are similar to those registered in other investigations, added to the fact that *Dunaliella tertiolecta* has a greater temperature tolerance range, biodiesel production will not be greatly affected.

By keeping the culture in open ponds for only one day, the probability of contamination of the culture is minimized, a problem that normally does not allow large volumes of production using this culture method.

Considering that the demand for liquid fuels in Peru is 284 thousand barrels per day (16,500.0 thousend tons per year), the theoretical production of biodiesel obtained (160 thousand tons per year) in this investigation, barely represents 0.98% of the national demand for 2020 and this same percentage of consumption applies to the year 2021, since the Peruvian economy is just being reactivated due to Covid-19. It is necessary to evaluate the capture of CO_2 emissions from other industrial plants located in regions with the ideal climatic conditions for the growth of microalgae in order to determine the total potential of Peru for the production of biodiesel from them.

References

[1] IEA, "World Energy Outlook 2012," International Energy Agency, Paris, 2012.

[2] U. E. I. Administration, "International Energy Outlook 2014," U.S. Energy Information Administration, Washington, 2014.

[3] IEA, "International Energy Agency," International Energy Agency, October 2019. [Online]. Available: https://www.iea.org/reports/renewables-2019/transport#abstract. [Accessed 28 November 2021].

[4] IRENA, "Global Energy Transformation: A Roadmap to 2050," IRENA International Renewable Energy Agency, Abu Dhabi, 2019.

[5] R. Araújo, F. Vázquez Calderón, J. Sánchez López, I. C. Azevedo, A. Bruhn, S. Fluch, M. Garcia Tasende, F. Ghaderiardakani, T. Ilmjärv, M. Laurans, M. Mac Monagail, S. Mangini, C. Peteiro, C. Rebours, T. Stefansson and J. Ullmann, "Current Status of the Algae Production Industry in Europe: An Emerging Sector of the Blue Bioeconomy," *Frontiers in Marine Science,* vol. 7, 2021.

[6] O. Acosta and A. Chaparro-Giraldo, "Biocombustibles, Seguridad Alimentaria y Cultivos Transgénicos," *Salud pública,* pp. 290-300, 2009.

[7] E. B. Sydney, W. Sturm, J. C. de Carvalho, V. Thomaz-Soccol, C. Larroche, A. Pandey and C. R. Soccol, "Potential carbon dioxide fixation by industrially important microalgae," *Bioresource Technology,* vol. 101, p. 5892–5896, 2010.

[8] O. f. E. C. Development, "OECD iLibrary," Organization for Economic Cooperation and Development, [Online]. Available: https://www.oecd-ilibrary.org/sites/8d79647e-es/index.html?itemId=/content/component/8d79647e-es. [Accessed 12 November 2021].

[9] E.-G. Abd Allah S. A., "Cost Analysis for Biodiesel Production from Waste Cooking Oil Plant in Egypt," *INTERNATIONAL JOURNAL of SMART GRID,* vol. 1, no. 1, pp. 16-25, 2017.

[10] E. S. Mostafa, N. Gholamhassan and N. Abbas, "The Biomass Supply Chain Network Auto-Regressive Moving Average Algorithm," *INTERNATIONAL JOURNAL of SMART GRID,* vol. 5, no. 1, pp. 15-22, 2021.

[11] S. Naseem, M. I. Abid, M. Kamran, M. R. Fazal, G. Abbas, M. R. Abid and Z. Zamir, "Rural Areas Interoperability Framework: Intelligent Assessment of Renewable Energy Security Issues in PAKISTAN," *INTERNATIONAL JOURNAL of SMART GRID,* vol. 4, no. 2, pp. 43-56, 2020.

[12] E. R. Aquino Larico, "Wind Energy Potential by the Weibull Distribution at High-Altitude Peruvian Highlands," *INTERNATIONAL JOURNAL of SMART GRID,* vol. 5, no. 3, pp. 113-120, 2021.

[13] F. Ayadi, I. Colak, I. Garip and H. I. Bulbul, "Impacts of Renewable Energy Resources in Smart Grid," *8th IEEE International Conference on Smart Grid,* pp. 183-188, 2020.

[14] A. Rios Villacorta, "Perú Modelo Energético Sostenible," 18 July 2020. [Online]. Available: https://albertorios.eu/?p=3043. [Accessed 27 November 2021].

[15] OSINERGMIN, "Reporte Semestral de Monitoreo del Mercado de Hidrocarburos," OSINERGMIN, Lima, 2020.

[16] MINEM, "Balance Nacional de energía 2018," MINEM, Lima, 2018.

[17] OSINERGMIN, "Demanda de combustibles líquidos (MBPD) total país," OSINERGMIN, Lima - Perú, 2021.

[18] OSINERGMIN, "OSINERGMIN," OSINERGMIN, Marzo 2021. [Online]. Available: https://www.osinergmin.gob.pe/empresas/hidrocarburos/Paginas/SCOP-DOCS/scop_docs.htm. [Accessed 28 November 2021].

[19] S. Mohite and S. Maji, "Importance of Energy Audit in Diesel Engine Fuelled with Biodiesel Blends: Review and Analysis," *European Journal of Sustainable Development Research,* vol. 4, pp. 2542-4742, 2020.

[20] S. Mohite and S. Maji, "Biofuel Certification Performance: A Review & Analysis," *European Journal of Sustainable Development Research,* vol. 4, pp. 2542-4742, 2020.

[21] A. Atabani, A. Silitonga, I. A. Badruddin, T. Mahlia, H. Masjuki and S. Mekhilef, "A comprehensive review on biodiesel as an alternative energy resource and its characteristics," *Renewable and Sustainable Energy Reviews,* vol. 16, pp. 2070-2093, 2012.

[22] P. J. l. B. Williams and L. M. L. Laurens, "Microalgae as biodiesel & biomass feedstocks: Review & analysis of the biochemistry, energetics & economics," *Energy & Environmental Science,* vol. 3, pp. 554-590, 2010.

[23] M. T. Arias Peñaranda, A. d. J. Martinez Roldan and R. O. Cañizares Villanueva, "Producción de Biodiesel a partir de Microalgas: Parámetros del cultivo que afectan la producción de lípidos," *Acta biol. Colomb.,* vol. 18, no. 1, pp. 43-68, 2013.

[24] A. Garibay Hernández, R. Vázquez-Duhalt, S. M. d. P. Sánchez, L. Serrano Carreón and A. Martínez Jiménez, "Biodiesel a Partir de Microalgas," *BioTecnología,* vol. 13, no. 3, 2009.

[25] É. C. Francisco, D. B. Neves, E. Jacob-Lopes and T. T. Franco, "Microalgae as feedstock for biodiesel production: Carbon dioxide sequestration, lipid production and biofuel quality," *Journal of Chemical Technology & Biotechnology,* vol. 85, p. 395–403, 2010.

[26] M. Aziz, T. Oda, T. Mitani, A. Uetsuji and T. Kashiwagi, "Combined Hydrogen Production and Power Generation from Microalgae," *4th International*

Conference on Renewable Energy Research and Applications, pp. 923-927, 2015.

[27] E. K. Çoban, C. Gençoglu, D. Kirman, O. Pinar, D. Kazan and A. A. Sayar, "Assessment of the effects of medium composition on growth, lipid accumulation and lipid profile of *Chlorella vulgaris* as a biodiesel feedstock," *4th International Conference on Renewable Energy Research and Applications,* pp. 793-796, 2015.

[28] T. M. Mata, A. A. Martins and N. S. Caetano, "Microalgae for biodiesel production and other applications: A review," *Renewable and Sustainable Energy Reviews,* vol. 14, no. 1, pp. 217-232, 2010.

[29] M. Kumar, Y. Sun, R. Rathour, A. Pandey, I. S. Thakur and D. C. Tsang, "Algae as potential feedstock for the production of biofuels and value-added products: Opportunities and challenges," *Science of the Total Environment,* vol. 716, 2020.

[30] B. G. Şanli, "Energetic and exergetic performance of a diesel engine fueled with diesel and microalgae biodiesel," *Energy Sources, Part A: Recovery, Utilization, and Environmental Effects,* vol. 41, no. 20, pp. 2519-2533, 2019.

[31] B. O. Arredondo and R. Vázquez, "Aplicaciones biotecnológicas en el cultivo de microalgas," *Ciencia y desarrollo,* vol. 27, no. 98, pp. 99-111, 1991.

[32] A. M. Santos, Y. González-Arechavala and C. Martín-Sastre, "Uso y aplicaciones potenciales de las microalgas," *Anales de Mecánica y Electricidad,* vol. 91, no. 1, pp. 20-28, 2014.

[33] D. L. Jaimes, W. Soler, J. Velasco, Y. Muñoz and N. A. Urbina, "Characterization *Chlorophyta* microalgae with potential in the production of lipid for biofuels," *Ciencia Tecnología y Futuro,* vol. 5, no. 1, pp. 93-102, 2012.

[34] J. L. Borja-Aragón, J. A. Rodríguez-De la Garza, L. J. Ríos-González, Y. Garza- García, M. M. Rodríguez-Garza and S. Y. Martínez-Amador, "Tratamiento de aguas residuales domésticas empleando *Chlorella vulgaris* en un biorreactor airlift," *Mexican journal of biotechnology,* vol. 2, no. 2, pp. 40-52, 2017.

[35] Y. Chisti, "Biodiesel from microalgae," *Biotechnology Advances,* vol. 25, pp. 294-306, 2007.

[36] J. N. Rosenberg, G. A. Oyler, L. Wilkinson and M. J. Betenbaugh, "A green light for engineered algae: redirecting metabolism to fuel a biotechnology revolution," *Current Opinion in Biotechnology,* vol. 19, no. 5, pp. 430-436, 2008.

[37] G. Knothe, "Dependence of biodiesel fuel properties on the structure of fatty acid alkyl esters," *Fuel Processing Technology,* vol. 86, p. 1059–1070, 2005.

[38] G. A. Cuevas-Castillo, F. S. Navarro-Pineda, S. A. Baz Rodríguez and J. C. Sacramento Rivero, "Advances on the processing of microalgal biomass for energy-driven biorefineries," *Renewable and Sustainable Energy Reviews,* vol. 125, p. 109606, 2020.

[39] J. Sheehan, T. Dunahay, J. Benemann and P. Roessler, "A Look Back at the U.S. Department of Energy's Aquatic Species Program—Biodiesel from Algae," National Renewable Energy Laboratory, Colorado, 1998.

[40] J. Seepratoomrosh, P. Pokethitiyook, M. Meetam, K. Yokthongwattana, W. Yuan, W. Pugkaew and K. Kangvansaichol, "The Effect of Light Stress and Other Culture Conditions on Photoinhibition and Growth of *Dunaliella tertiolecta*," *Applied Biochemistry and Biotechnology,* vol. 178, no. 2, pp. 396-407, 2015.

[41] Global Solar Atlas, "Global Solar Atlas," [Online]. Available: https://globalsolaratlas.info/detail?c=-4.605172,-81.220207,11&s=-4.580532,-81.280975&m=site. [Accessed 25 November 2021].

[42] A. Rios Villacorta, "Proyecto Yuyo," 2012.

[43] J. Clippinger and R. Davis, "Techno-Economic Analysis for the Production of Algal Biomass via Closed Photobioreactors: Future Cost Potential Evaluated Across a Range of Cultivation System Designs," National Renewable Energy Laboratory, Denver, 2019.

[44] L. Brennan and P. Owende, "Biofuels from microalgae—A review of technologies for production, processing, and extractions of biofuels and co-products," *Renewable and Sustainable Energy Reviews,* vol. 14, no. 2, pp. 557-577, 2010.

[45] A. Richmond, Handbook of Microalgal Culture, Carlton, Victoria 3053, Australia: Blackwell Science Ltd a Blackwell Publishing company, 2004.

[46] O. Pulz and K. Scheibenbogen, "Photobioreactors: Design and performance with respect to light energy input," *Advances in Biochemical Engineering Biotechnology,* pp. 123-152, 1998.

[47] O. Pulz, "Photobioreactors: production systems for phototrophic microorganisms," *Applied Microbiology and Biotechnology,* vol. 57, no. 3, pp. 287-293, 2001.

[48] Y. Li, M. Horsman, N. Wu, C. Lan and N. Dubois-Calero, "Biofuels from Microalgae," *Biotechnology Progress,* 2008.

[49] B. E. Rittmann, "Opportunities for renewable bioenergy using microorganisms," *Biotechnology and Bioengineering,* vol. 100, no. 2, pp. 203-212, 2008.

[50] P. M. Schenk, S. R. Thomas-Hall, E. Stephens, U. C. Marx, J. H. Mussgnug, C. Posten, O. Kruse and B. Hankamer, "Second Generation Biofuels: High-Efficiency Microalgae for Biodiesel Production," *BioEnergy Research,* vol. 1, no. 1, pp. 20-43, 2008.

[51] R. H. Wijffels, "Potential of sponges and microalgae for marine biotechnology," *Trends in Biotechnology,* vol. 26, no. 1, pp. 26-31, 2008.

[52] A. Dahiya, "Algae Biomass Cultivation for Advanced Biofuel Production," *Bioenergy,* pp. 219-238, 2015.

[53] H.-Y. Ren, B.-F. Liu, F. Kong, L. Zhao, G.-J. Xie and N.-Q. Ren, "Enhanced lipid accumulation of green microalga *Scenedesmus sp*. by metal ions and EDTA addition," *Bioresource Technology,* vol. 169, pp. 763-767, 2014.

[54] W. Zhang, Y. Zhao, B. Cui, H. Wang and T. Liu, "Evaluation of filamentous green algae as feedstocks for biofuel production," *Bioresource Technology,* vol. 220, pp. 407-413, 2016.

[55] E. Molina Grima, E.-H. Belarbi, F. Acién Fernández, A. Robles Medina and Y. Chisti, "Recovery of microalgal biomass and metabolites: process options and economics," *Biotechnology Advances,* vol. 20, no. 7-8, pp. 491-515, 2003.

[56] J. García, M. Hernández-Mariné and R. Mujeriego, "Influence of Phytoplankton Composition on Biomass Removal from High-Rate Oxidation Lagoons by Means of Sedimentation and Spontaneous Flocculation," *Water Environment Research,* vol. 72, no. 2, pp. 230-237, 2000.

[57] H. C. Greenwell, L. M. L. Laurens, R. J. Shields, R. W. Lovitt and K. J. Flynn, "Placing microalgae on the biofuels priority list: a review of the technological challenges," *Journal of The Royal Society Interface,* vol. 7, no. 46, pp. 703-726, 2009.

[58] R. Muñoz and B. Guieysse, "Algal–bacterial processes for the treatment of hazardous contaminants: A review," *Water Research,* vol. 40, no. 15, pp. 2799-2815, 2006.

[59] R. M. Knuckey, M. R. Brown, R. Robert and D. M. Frampton, "Production of microalgal concentrates by flocculation and their assessment as aquaculture feeds," *Aquacultural Engineering,* vol. 25, no. 3, pp. 300-313, 2006.

[60] L. Bermeo Castillo, "Estudio del cosechado de cultivos de microalgas en agua residual mediante técnicas de centrifugado," Universidad Técnica Particular de Loja, 2011.

[61] M. Heasman, J. Diemar, W. O'connor, T. Sushames and L. Foulkes, "Development of extended shelf-life microalgae concentrate diets harvested by centrifugation for bivalve molluscs - a summary," *Aquaculture Research,* vol. 31, no. 8-9, pp. 637-659, 2000.

[62] M. Aziz, T. Oda and T. Kashiwagi, "Energy-Efficient Algae Utilization Based on Enhanced Process Integration," *International Conference on Renewable Energy Research and Applications,* pp. 395-400, 2013.

[63] J. Kim, G. Yoo, H. Lee, J. Lim, K. Kim, C. W. Kim, M. S. Park and J.-W. Yang, "Methods of downstream processing for the production of biodiesel from microalgae," *Biotechnology Advances,* vol. 31, no. 6, pp. 862-876, 2013.

[64] G. Batista, G. A. Surek, C. Benincá, M. L. Corazza and E. F. Zanoelo, "Cyclic pressurization assisted extraction of lipids from microalgae for biodiesel production: Non-equilibrium and equilibrium data," *Fuel,* vol. 163, pp. 133-138, 2016.

[65] H. Taher, S. Al-Zuhair, A. H. Al-Marzouqi, Y. Haik and M. Farid, "Effective extraction of microalgae lipids from wet biomass for biodiesel production," *Biomass and Bioenergy,* vol. 66, pp. 159-167, 2014.

[66] C. Barraza, V. Collao, C. Espinoza, F. Moya, G. Thun and M. Torres, "Producción de biodiesel a partir de microalgas," Pontificia Universidad Católica de Valparaiso, Valparaiso, 2009.

[67] D. M. Guimarães Freire, J. S. de Sousa and E. d. Cavalcanti-Oliveira, "Biotechnological Methods to Produce Biodiesel," *Biofuels,* pp. 315-337, 2011.

[68] R. Kwangdinata, I. Raya and M. Zakir, "Production of Biodiesel from Lipid of Phytoplankton *Chaetoceros calcitrans* through Ultrasonic Method," *The Scientific World Journal,* vol. 2014, pp. 1-5, 2014.

[69] E. H. Durán-Bautista, Y. K. Ángel-Sánchez and J. C. Suárez-Salazar, "Dinámica de la radiación fotosintéticamente activa en arreglos agroforestales con *Hevea brasiliensis* en el norte de la amazonía colombiana," *Revista U.D.C.A Actualidad & Divulgación Científica,* vol. 18, no. 2, pp. 365-372, 2015.

[70] E. M. Grima, F. G. Camacho, J. A. S. Pérez, J. M. F. Sevilla, F. G. A. Fernández and A. C. Gómez, "A mathematical model of microalgal growth in light-limited chemostat culture," *Journal of Chemical Technology & Biotechnology,* vol. 61, no. 2, pp. 167-173, 1994.

[71] M. T. Myint, A. Ghassemi and N. Nirmalakhandan, "A generic stoichiometric equation for microalgae–microorganism nexus by using clarified domestic wastewater as growth medium," *Desalination and Water Treatment,* vol. 51, no. 34-36, pp. 6632-6640, 2013.

[72] S. Judd, L. J. van den Broeke, M. Shurair, Y. Kuti and H. Znad, "Algal remediation of CO_2 and nutrient discharges: A review," *Water Research,* vol. 87, pp. 356-366, 2015.

[73] I. Rawat, R. Ranjith Kumar, T. Mutanda and F. Bux, "Biodiesel from microalgae: A critical evaluation from laboratory to large scale production," *Applied Energy,* pp. 444-467, 2013.

[74] A. Richmond and Q. Hu, Handbook of Microalgal Culture: Applied Phycology and Biotechnology, Wiley, 2013.

[75] M. E. Huntley and D. G. Redalje, "CO_2 Mitigation and Renewable Oil from Photosynthetic Microbes: A New Appraisal," *Mitigation and Adaptation Strategies for Global Change,* vol. 12, no. 4, pp. 573-608, 2006.

[76] M. R. Tredici, "Mass Production of Microalgae: Photobioreactors," *Handbook of Microalgal Culture,* pp. 178-214, 2007.

[77] G. C. Zittelli, L. Rodolfi, N. Bassi, N. Biondi and M. R. Tredici, "Photobioreactors for Microalgal Biofuel Production," *Algae for Biofuels and Energy,* pp. 115-131, 2012.

[78] M. Chen, H. Tang, H. Ma, T. C. Holland, K. S. Ng and S. O. Salley, "Effect of nutrients on growth and lipid accumulation in the green algae *Dunaliella tertiolecta*," *Bioresource Technology,* vol. 102, no. 2, pp. 1649-1655, 2011.

[79] A. Hosseini Tafreshi and M. Shariati, "*Dunaliella* biotechnology: methods and applications," *Journal of Applied Microbiology,* vol. 107, no. 1, pp. 14-35, 2009.

[80] Benefuel Inc., "Benefuel," Benefuel Inc., 2021. [Online]. Available: http://www.benefuel.net/technology.php. [Accessed 12 November 2021].