

# Characterization and validation of a new photobioreactor, bubble column type with axial mixing, for production of single cell oil for biodiesel from microalgae

Mariana Simões de Araújo

*Instituto Superior Técnico, Lisbon, Portugal*

May 2014

---

## Abstract

In the last decades, intensive utilization of fossil fuels has led to energy crisis, global climate change and environmental pollution. Although there are various potential sources of renewable energy, biofuels are of most interest and are expected to play a crucial role in the global energy infrastructure in the future. Among the available feedstocks for the production of biofuels, whose competition with edible vegetable oil for agricultural land is still a controversial issue, microalgae have been described as the most sustainable alternative feedstock for the next generation of biodiesel production. The aim of this work was the characterization and validation of a new photobioreactor for the cultivation of microalgae for biodiesel production. The reactor was bubble column type and the sparger was a circular sparger placed in the centre of its base. The sparger is rotatable, being the rotations dependent of the flow rate of injected gas.

The first part of this work was the determination of the photobioreactor characterization parameters. The highest rotation of the sparger was reached, for a volume of 50 L, with a gas flow rate of 22,9 L/min, and was 2,2 rpm. Concerning to hold up, the maximum values were registered for the highest gas speeds and for the least liquid level in the reactor. The obtained values are low. This could be due to the bubble size, which strongly depends on the sparger type used. The smaller the bubbles, the greater the gas hold up values. For mixing time, the trend was a declining mixing time with increasing gas flow rate. The shorter mixing time was obtained for the gas flow rate of 22,9 L/min and was 33,9 s, for the dynamic sparger. It was found that for higher flow rates the rotation of the sparger, although higher, makes no improvement in the mixing time. The  $k_L a$  increases with increasing gas flow rate for all operating conditions. It was verified a tendency for higher  $k_L a$  values in the case of the dynamic sparger that, although it might be within the measuring error, seems to indicate advantage of the sparger rotation in mass transfer. The higher  $k_L a$  value was obtained for the gas flow rate of 22,9 L/min and was  $0,011 \text{ s}^{-1}$ .

The *Scenedesmus obliquus* cultivation was successful and lasted 14 days. The results of the daily follow-up of both cultures did not show evident differences between the photobioreactor with the static sparger and the one with the dynamic sparger. It can be concluded that the photobioreactor studied is adequate for the growth of microalgae needing, however, the optimization of the experimental conditions in order to maximize the biomass production and lipid accumulation.

Finally, it was used an industrial anodising sludge and was studied its capacity as coagulant of a microalgae broth, as a first step of biomass harvesting. It was verified some flocculation capacity of the tested sludge but the flocculation conditions need further optimization.

**Keywords:** *Scenedesmus obliquus*, photobioreactor, biodiesel, hold up, mixing time, volumetric oxygen transfer coefficient, lipids, fatty acids, flocculation

---

## 1. Introduction

Nowadays, fossil fuels account for over 80% of the primary energy consumed in the world [1]. The need for security and diversification of energy supply, the necessity for independence from fossil fuels, the uncertainty surrounding oil prices and the raising concerns over environmental degradation and climate change effects are some of the reasons why many countries are turning their attention on the promotion of renewable energy sources. The use of biomass, particularly biofuels, for energy purposes, becomes increasingly interesting [2]. Obtained from natural sources, biofuels are renewable and can recycle the  $\text{CO}_2$  from their combustion through photosynthesis. They are produced from agricultural products such as sugarcane, oleaginous plants and forest biomass. Biofuel production from crops is criticised for increases in food prices and food insecurity, and the land use changes and intensification of cultivation following the

increasing demand for biofuels may promote new greenhouse gas emissions and affect the biodiversity, the soil quality and the natural resources of the region [3].

Microalgae have long been recognized as potentially good sources for biodiesel production. Biofuel production using microalgae biomass offers many advantages [3], [4]: (1) microalgae can be cultivated in brackish water on non-arable land and, therefore, may not incur land-use change, minimising associated environmental impacts, while not compromising the production of food and other products derived from crops; (2) microalgae are capable of all year round production, therefore, oil productivity of microalgae cultures exceeds the yield of the best oilseed crops; (3) microalgae growth is extremely rapid and many algae species are rich in oils, and; (4) the tolerance of microalgae to high  $\text{CO}_2$  content in gas streams allows high-efficiency  $\text{CO}_2$  mitigation. However, microalgae as a feedstock for biodiesel

are currently more expensive than traditional agricultural crops and further R&D is required to reach the market.

Microalgae production based on closed photobioreactor technology is designed to overcome some of the major problems associated with open pond production systems. Photobioreactors allow culture of single-species of microalgae for prolonged durations with lower risk of contamination, reproducible cultivation conditions, controllable hydrodynamics and temperature. However, the costs of closed systems are substantially higher than open pond systems [5], [6].

Bubble columns reactors owe their wide application area to a number of advantages they provide, both in design and operation, as compared to other reactors and have been advanced as effective photobioreactors for large-scale culture of microalgae. They have excellent heat and mass transfer characteristics and required little maintenance and low operating costs due to lack of moving parts and compactness [7]. Recent research with bubble columns frequently focuses on gas holdup studies, flow regime investigations, heat and mass transfers and mixing time.

Gas holdup is a dimensionless key parameter for design purposes that characterizes transport phenomena of bubble column systems. It is defined as the volume fraction of gas phase occupied by the gas bubbles [7], [8].

The oxygen mass transfer coefficient is a key parameter in the characterization and design of bubble column systems. Most investigations performed are limited to the determination of the volumetric mass transfer coefficient,  $k_L a$ , which is the product of the liquid mass transfer coefficient,  $k_L$ , and interfacial area,  $a$  [7], [9].

Mixing time,  $t_m$ , is the time required to attain a given deviation from the fully mixed state from the instance of a tracer input [10].

Effective supply of light and  $\text{CO}_2$  to the whole cell culture, as well as other physical and nutritional requirements are essential for microalgae growth [11].

Light is the basic energy source for autotrophic organisms. High light intensities combined with low biomass concentrations could be harmful to photosynthetic cultures due to photo-inhibition and photo-oxidation. Low light intensities and/or high biomass concentrations increase endogenous biomass consumption by respiration.

Mixing is another important growth parameter since it produces a uniform dispersion of microalgae within the culture medium, thus eliminating gradients of light, nutrient concentration and temperature. Also, a certain degree of turbulence is desirable in order to promote the fast circulation of microalgae cells from the dark to the light zone of the reactor. On other hand high liquid velocities and degrees of turbulence (due to mechanical mixing or air bubbles mixing) can damage microalgae due to shear stress [5].

Continuous removal of oxygen is essential, as excessive dissolved oxygen in the broth inhibits photosynthesis.

Harvesting of biomass requires one or more solid-liquid separations steps. This is considered to be an expensive and problematic part of industrial production of microalgal biomass. Biomass can be harvested by centrifugation, filtration or in some cases, gravity sedimentation. These processes may proceed by a flocculation step [12].

The aim of this work is the characterization and validation of a new photobioreactor, bubble column type, for the cultivation of microalgae for biodiesel production. In this work it is also intended to use an industrial anodising sludge

and study its capacity as coagulant of a microalgae broth, as a first step of biomass harvesting.

## 2. Materials and Methods

### 2.1. Determination of the photobioreactor characterization parameters

Experiments were carried out on a vertical bubble column made of acrylic. The column was 0,22 m in diameter and 2 m in height, with a maximum working volume of 0,07 m<sup>3</sup>. The liquid was tap water and the gas used for all experiments was air. The sparger was circular with three rotating arms with five holes each. The diameter of the holes was 2 mm.

#### 2.1.1 rpm vs. gas flow rate

It was measured the time needed for a complete rotation of the sparger. Gas flow rates ranged from 8,3 to 29,2 L/min. It was tested the volumes of liquid of 50, 60 and 65 L.

#### 2.1.2 Gas hold-up

Gas hold-up was determined by measuring the aerated liquid height relative to the gas-free liquid level.

#### 2.2.3 Mixing time

The pH tracer method was used to measure the mixing time, defined as the time required to attain a 5% deviation from complete homogeneity from the instance of tracer addition. The pH tracer (HCl (2M) or NaOH (2M)) was added at the centre of the surface of the liquid. The change in pH with time was measured using a pH electrode located on the liquid surface. Gas flow rates ranged from 5,8 to 22,9 l/min, for a working volume of 50 L. Mixing time was measured in two situations: i) static sparger with the holes vertically oriented, and ii) dynamic sparger with the holes horizontally oriented.

#### 2.2.4 Oxygen mass transfer coefficient

The oxygen mass transfer coefficient,  $k_L a$ , was measured using the dynamic gassing-out method. For  $k_L a$  measurements, dissolved oxygen was first removed from the reactor by sparging with nitrogen until the dissolved oxygen concentration approached zero. Time was given to allow the  $\text{N}_2$  bubbles to disengage and then air was sparged at the required rate. The dissolved oxygen concentration ( $C$ ) versus time ( $t$ ) was recorded until close to saturation. For the procedure described, the following equation holds,

$$\frac{dC_L}{dt} = K_L a (C^* - C_L)$$

Integration for  $C_L=C_0$  at  $t=0$ , led to Eq. (2).

$$\ln\left(\frac{C^* - C_L}{C^* - C_0}\right) = -K_L a \cdot t$$

A plot of the left hand side of this equation against time was used to obtain  $-k_L a$  as the slope.

$k_L a$  was measured for the two situations described in 2.2.3 and for the same gas flow rates.

## 2.2 Microalgae cultivation

### 2.1. Microalgal strain

The microalgal strain used in this study was *Scenedesmus obliquus* from FCTU Coimbra.

### 2.2. Medium

*Scenedesmus* was cultivated in an inorganic medium containing per liter: 0.25 g NaNO<sub>3</sub>, 0.175 g KH<sub>2</sub>PO<sub>4</sub>, 0.075 g MgSO<sub>4</sub>·7H<sub>2</sub>O, 0.033 g CaCl<sub>2</sub>·2H<sub>2</sub>O, 0.025 g NaCl, 0.075 g K<sub>2</sub>HPO<sub>4</sub>, 0.1 mg FeEDTA·3H<sub>2</sub>O, and 1 mL trace elements solution. The trace elements solution contained per liter: 2.86 g H<sub>3</sub>BO<sub>3</sub>, 1.54 g MnSO<sub>4</sub>·H<sub>2</sub>O, 220 mg ZnSO<sub>4</sub>, 50 mg CuSO<sub>4</sub>, 60 mg Na<sub>2</sub>MoO<sub>4</sub>·2H<sub>2</sub>O and 80 mg CoSO<sub>4</sub>·6H<sub>2</sub>O.

### 2.3. Cultivation

*Scenedesmus obliquus* was grown in two photobioreactors described in 2.1, with a working volume of 50 L, under artificial light (24h/day) and atmospheric air inflow of 22,9 L/min. Each photobioreactor was filled with 47,5 L of the medium and 2,5 L of inoculum. Its inoculum was obtained from *Scenedesmus* grown in flasks under artificial light (24h/day) and atmospheric air inflow between 1 and 4 L/min.

In one photobioreactor the sparger had no rotation and the holes were vertically oriented (static sparger). In the other the sparger had rotation and the holes were horizontally oriented (dynamic sparger).

The liquid volume was completed to 50 L daily to account for the evaporation.

During *Scenedesmus* growth the pH was monitored via the specific electrode SZ12T (Consort Multi-parameter C3021). Samples were taken in both photobioreactors during *Scenedesmus* growth, to analyse several parameters.

### 2.4 Flocculation tests

The anodizing mud was collected in a Portuguese facility having the operations of aluminium anodizing. The sludge came from the wastewaters treatment of the anodizing plants and was collected at the press-filter discharge. Aluminium is one of the major constituents of the composition of the solid fraction along with the total sulphur. The total solids in the mud suspensions tested were 5%.

In this study was used a culture of *Chlorella protothecoides*.

A series of tests was programmed, being controlled important parameters such as flocculation dose and mixing and flocculation conditions (stirring time and speed and settling time). The laboratorial tests were performed using samples of 1 L of *Chlorella protothecoides* broth. Turbidity measurements represent a convenient experimental procedure for the determination of the stability of colloidal suspensions. As aggregation occurs and the colloids settle out of solution, turbidity decreases.

The flocculation conditions used in the tests are indicated in Table 1.

Table 1 – Flocculation conditions.

Stirring Conditions	
Rapid Stirring Speed	120, 150 or 200 rpm
Rapid Stirring time	5 or 15 min
Slow Stirring Speed	45, 60 or 90 rpm
Slow Stirring time	10, 30 or 60 min
Sedimentation time	30 or 60 min
Flocculant dose	0,2, 0,5 or 1 g/L

### 2.5. Analytical methods

#### 2.5.1. Growth analysis

The growth of *Scenedesmus* was monitored by determination of dry weight (DW) after filtering a known volume culture medium with a glass fibre filter and drying 18 h at 100°C and by measuring the optical density at 540nm [13]. Both measurements were made in duplicate.

#### 2.5.2. Nitrate determination

The ultraviolet spectrophotometric method was used to analyse nitrate in the medium after cells separation [14].

#### 2.5.6. Determination of fatty acid content and composition

Fatty acid composition of *Scenedesmus* was analysed by gas chromatography. The fatty acids were transesterified by Lepage and Roy's method [15] with modifications for GC analysis. To a sample of *Scenedesmus* powder (100 mg) 2 ml of a mixture of methanol/acetyl chloride (95:5 v/v) and 0,2 mL of internal standard solution from heptadecanoic acid in petroleum benzin 60-80°C (5 mg/ml) were added.

The mixture was sealed in a light-protected vial under nitrogen atmosphere and heated at 80°C for 1 h. After cooling, the vial contents were diluted with 1 ml of water and 2 ml of n-heptane. After 15 min, the upper layer (heptanoic phase) was recovered, then dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>, filtered and collected in a vial, obtaining the fatty acid methyl esters. These were analysed by gas chromatography in a Scion 436-GC chromatograph (Bruker, USA), equipped with a flame ionization detector (FID). Separation was carried out in a 0.32 mm × 30 m fused silica capillary column (film 0.32 µm) Supelcowax 10 with helium as carrier gas at a flow rate of 28 ml/min. The column temperature was programmed at an initial temperature of 200°C for 1 min, then increased at 2,5°C/min to 240°C and held at 240°C for 10 min. Injector and detector temperatures were maintained at 250°C. Each sample was prepared in duplicate.

#### 2.5.7 Productivity determination

The partial biomass productivity was calculated as

$$P_x = \frac{m_i - m_0}{\left(\frac{V_i + V_0}{2}\right) \times (t_i - t_0)}$$

the global biomass productivity was calculated as

$$P_{Xtot} = \frac{m_f - m_{ini}}{V_f \times t}$$

and the lipid productivity was calculated as

$$P_{FA} = P_{Xtot} \times (\%FA/100)$$

where  $m_i$  and  $V_i$  are the total biomass and culture volume, respectively, after a period of time  $t_i$ ;  $m_0$  and  $V_0$  the total biomass and culture volume, respectively, measured before  $t_i$  ( $t_0$ );  $m_f$  and  $V_f$  are the biomass and culture volume, respectively, at the end of the experiment;  $m_{ini}$  is the biomass at the beginning of the experiment;  $t$  is the total experiment time;  $\%FA$  is the biomass fatty acid content at the end of the experiment.

### 3. Results and discussion

#### 3.1. Determination of the photobioreactor characterization parameters

##### 3.1.1 rpm vs. gas flow rate

A similar behaviour is observed for the different levels of liquid in the reactor reaching the highest rotation at the maximum injected air flow (Figure 1).

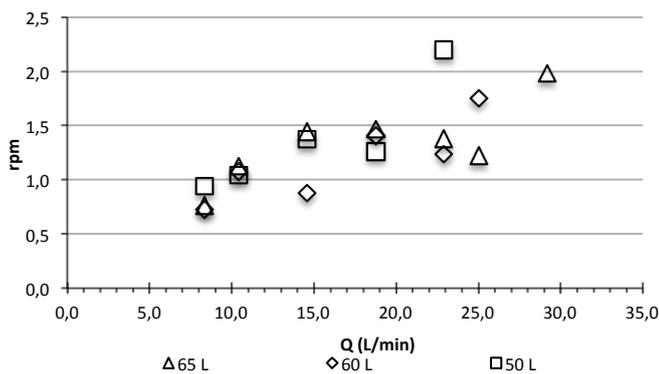


Figure 1 – rpm vs gas flow rate, for the volumes of liquid of 50, 60 and 65 L.

The highest rotation was reached, for a volume of 50 L, with a gas flow rate of 22,9 L/min. The highest the volume the highest the water mass and thus the resistance to motion.

This happens because for low gas flow rates the weight of the liquid column is lowest where the bubbles go up, the holes side, making the sparger to rotate in that direction. For moderate gas flow rates the kinetic energy of the gas at the hole exit is enough to balance the difference in weight in the liquid columns, keeping the sparger stopped, with eventual small displacements in one or other directions. For high gas flow rates the sparger rotates in the direction opposite to the gas exit.

##### 3.1.2 Gas hold-up

As expected, gas hold up increased when gas velocity increased (Figure 2).

The maximum hold up values were registered for the highest gas velocities and for the least liquid level in the reactor. The obtained values are low, what could be due to the

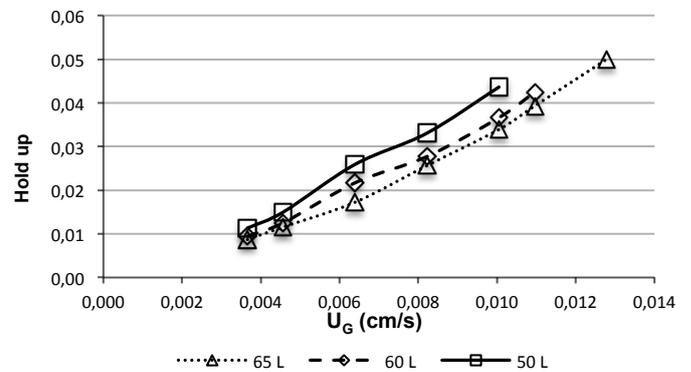


Figure 2 – Hold up vs gas velocity, for the volumes of liquido f 50, 60 and 65 L.

bubble size which strongly depends on the sparger type used. The smaller the bubbles, the greater the gas hold up values.

##### 3.2.3 Mixing time

Mixing times versus gas flow rate data are shown in Figure 3. In both situations, static and dynamic sparger, the trend was a declining mixing time with increasing gas flow rate. However, mixing time was shorter with the dynamic sparger.

It was verified that with the increase of air flow the mixing time of the dynamic and static spargers approach, being the same at the maximum flow. This leads to the possible conclusion that with low flow, thus with few bubbles, the slow rotation of the sparger has positive effects on the reactor's mixing. On the other hand for higher flows, thus with more bubbles and higher coalescence, the rotation of the sparger, although higher, makes no improvement in the mixing time due to the already significant turbulence in the system.

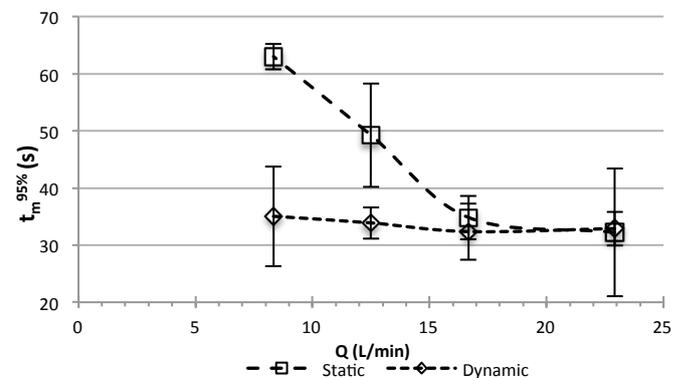


Figure 3 – Mixing time vs gas flow rate, for the static and dynamic spargers.

The obtained values present a high error considering that the chosen method was not the most adequate and that the pH electrode was at the surface of the liquid in the reactor where the pH tracer was added. This can lead to a situation where the upper part of the reactor has an apparently stable pH while the lower section is still in mixing phase.

##### 3.1.4 Oxygen mass transfer coefficient

In Figure 4, the oxygen mass transfer coefficient  $k_L a$  is reported versus the gas flow rate. The  $k_L a$  increases with increasing gas flow rate, for all operating conditions.

The observed increase in  $k_{La}$  values is higher when the sparger has rotation. Although the observed difference might be within the measuring error it seems to indicate a tendency for higher  $k_{La}$  values revealing thus advantages of the sparger rotation in the mass transfer.

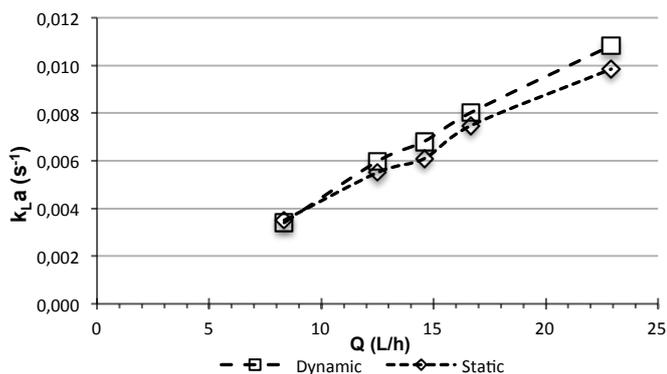


Figure 4 – Oxygen mass transfer coefficient vs gas flow rate, for the static and dynamic sparger.

### 3.2 Microalgae cultivation

The second part of this work was the validation of the photobioreactor for the cultivation of microalgae.

The growth of *Scenedesmus obliquus* under the tested conditions was successful and no contamination was detected during the growth. *Scenedesmus obliquus* reached its highest biomass concentration of 1,8 e 2,0 DW/L on the last day (day 14), in the photobioreactor with static sparger and dynamic sparger, respectively.

After 167 hours a 10 L volume of the culture was removed from each of the photobioreactors and replaced with the same volume with Bristol medium, 5 times concentrated, to re-establish the level of nitrates.

The specific growth rates were 0,033 and 0,032  $h^{-1}$ , in the photobioreactor with static sparger and dynamic sparger, respectively. After the dilution of the culture, *Scenedesmus* registered a lower growth rate value than the one previously observed, of 0,0065 e 0,0048  $h^{-1}$ , in the photobioreactor with static sparger and dynamic sparger, respectively. Since it was added the medium, corresponding to 50 L, to each reactor the decrease in growth rate cannot be due to the lack of nutrients. This decrease might be justified by light limitation, since being the culture very dense the light penetration was more difficult, and/or by limitation of carbon dioxide, since its level decreases

as it is consumed by denser culture.

The global biomass productivity was 0,128 and 0,134  $g/(L \cdot day)$ , in the photobioreactor with static sparger and dynamic sparger, respectively.

Comparing the microalgae growth in both reactors, with static sparger and dynamic sparger, we could not observe clear evidences of the benefit of using the last one.

It was verified that the growth rates were very close as well as the global biomass productivities.

Regarding the total biomass obtained, the photobioreactor with the dynamic sparger recorded a slightly higher value being, however, very close to the obtained on the photobioreactor with static sparger.

### 3.7. Fatty acid composition of microalgae oil

The total cellular fatty acid content was determined for the first days of growth.

Regarding the photobioreactor with static sparger, we can see that the lipidic content varies from 7.0 to 9.9% (Figure 6). This is the expected behaviour reaching its maximum by 163 hours (day 7) when the medium was limited in nitrate. This behaviour is verified in several studies that demonstrate that many microalgae species reach their maximum lipidic content under nitrogen deficiency because its absence produces lipid accumulation.

The photobioreactor with dynamic sparger has a similar behaviour with the exception of the 163 hours point (day 7). It would be expectable that it would present a higher value since, as previously said, when subjected to conditions of nutritional limitations, the algae produce reserve lipidic materials as a survival mechanism. The lipid contents are slightly lower than the previous case being between 4.0 and 9.8%.

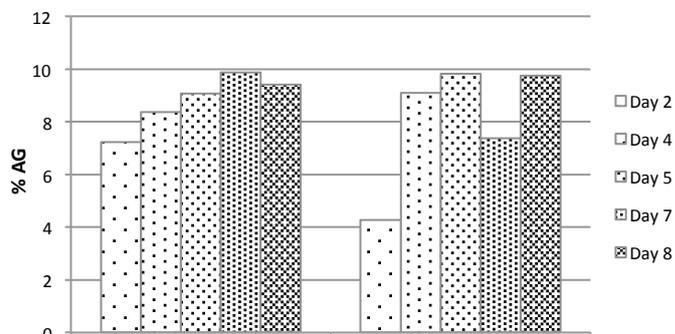


Figure 6 – Fatty acids content for static and dynamic spargers.

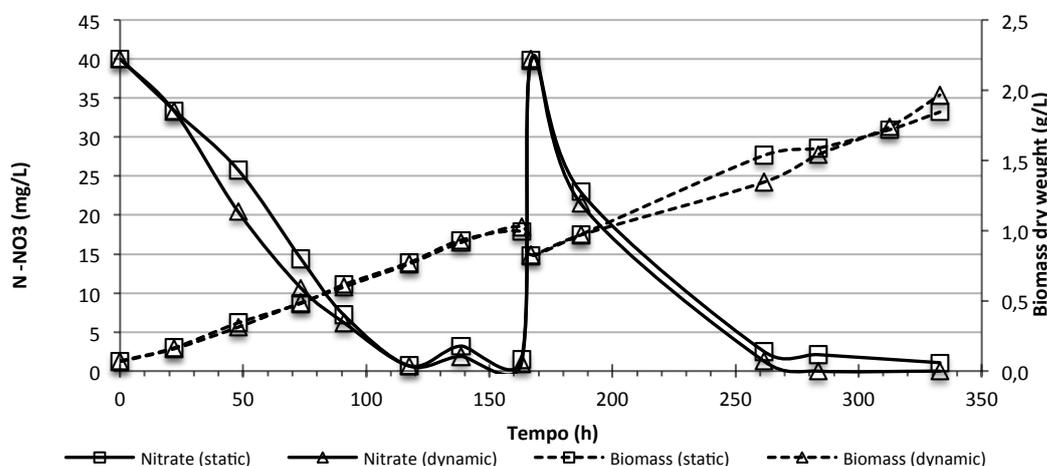


Figure 5 – Biomass and  $NO_3^-$ , for both static and dynamic sparger.

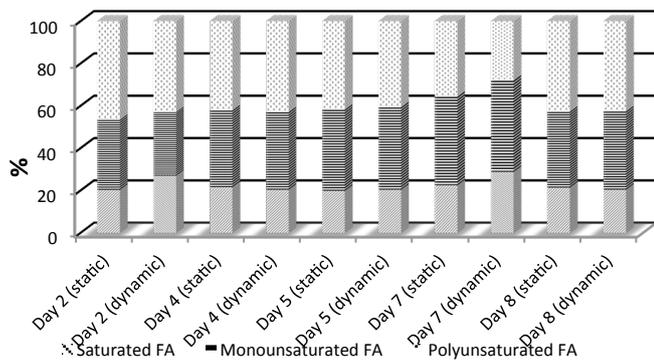


Figure 7 – Fatty acid composition for static and dynamic spargers.

The oil from *Scenedesmus obliquus* cannot be used directly as raw material for biodiesel, since does not complies the specification of the European Standard EN 14214 [16] that limits linolenic acid methyl ester to 12% (w/w) for biodiesel vehicle use. It requires an intermediate process (like blending with other oils or hydrogenation of the oil) to overcome the limitations and to be used for biodiesel production.

Predictions for biodiesel properties from the fatty acid composition can be made according to Ramos et al. [17] when subjected to a transesterification process. This study demonstrates that the fundamental properties of biodiesel correspond to the cetane number, oxidation stability, iodine value and cold filter plugging, which directly depends on the nature of oil. The researchers found that oils having more than 50% of monounsaturated FA, 20% or less of saturated FA and 30% or less of polyunsaturated FA, produced quality biodiesel which in turn complied with the limits imposed by the European Standard EN 14214 for critical parameters.

The oil from microalgae from both photobioreactors did not have a suitable composition for biodiesel production, since both cultivations (with static and dynamic sparger) exceed the saturation (20-29%) and/or polyunsaturation content (28-46%), and fall short of monounsaturatation content (30-43%).

#### 2.4 Flocculation tests

The initial phase of the coagulation process is the rapid mixing. The coagulant species causing destabilization are transported by turbulent eddies which interact with the particles in the fluid by collisions. The rapid mixing step is then followed by a period of less intense agitation where floc growth takes place up to sizes suitable for removal.

A series of tests was programmed, being controlled important parameters such as coagulant dose and mixing and flocculation conditions (stirring time and speed and settling time).

Flocculant dose is a very important parameter regarding the flocculation process efficiency. Different flocculation doses were tested (0,2 g/L to 1 g/L) in order to establish the more favourable conditions. This dosing interval was selected based on previously studies in which the same mud was used as flocculant.

Table 2 – Flocculation conditions and results for the four best tests selected based on the turbidity reduction.

Test	Flocculant Dose (g/L)	Rapid stirring speed (rpm)	Rapid stirring time (min)	Slow stirring speed (rpm)	Slow stirring time (min)	Sedimentation time (min)	Turbidity reduction (%)
14	0,5	150	5	45	60	60	93,5
15	1	150	5	90	30	60	97,6
16	1	120	15	45	30	60	93,6
18	0,5	120	5	60	60	60	93,6

The maximum turbidity reduction obtained with different flocculant doses is indicated in Figure 8. The maximum turbidity removal achieved with a flocculant dose of 0,2 g/L oscillated from 80 to 91%. With a flocculant dose of 0,5 g/L the maximum turbidity removal achieved ranged from 76 e 94 %. For the case of the higher dose of 1 g/L the turbidity removal reached 89 to 97,5%. So, increasing flocculant dose improves turbidity removal.

Table 2 shows the conditions and results for the four best tests selected based on the turbidity reduction.

Although the highest removal was achieved with the dose of 1 g/L, a dose of 0,5 g/L allows already good results for microalgae harvesting, as can be seen in Table 2.

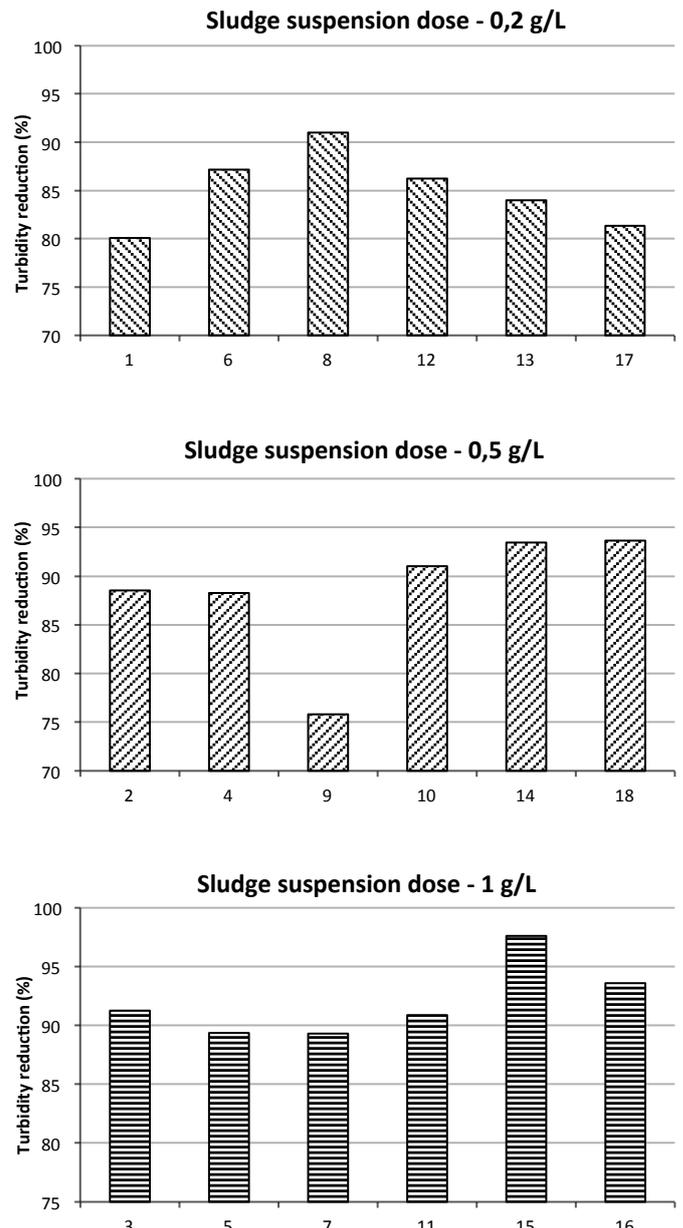


Figure 8 – Maximum turbidity reduction obtained with different flocculant doses.

Regarding the sedimentation time we can observe that the best results were sedimenting for 60 minutes. This was expected since, for the same conditions, the longer the sedimentation time the bigger the number of cells that sediment and, as a consequence, the higher the number of collected cells.

The flocculant dose was the decisive parameter regarding the biomass harvesting. However different stirring conditions were also tested. The highest percentages in turbidity reduction were not all achieved for the same stirring conditions. We can obtain ideal intervals of rapid stirring speeds and times between 120 and 150 rpm and 5 and 15 minutes, respectively, and slow stirring speeds and times between 45 and 90 rpm and 30 and 60 minutes, respectively. These conditions must be tested in further studies in order to obtain the optimized flocculation conditions in this microalgae species.

Nevertheless in order to select the more adequate conditions for biomass harvesting an equilibrium must be chosen between turbidity reduction and flocculation dose. It is necessary to assure that the added dosing does not compromise the final microalgae use – biodiesel production.

#### 4. Conclusions and future work

This study aimed to characterize and validate a new photobioreactor for the cultivation of microalgae for biodiesel production. The reactor was bubble column type and the sparger was circular and was placed in the centre of its base. The sparger is rotatable, being the rotation speed dependent of the flow rate of injected gas. This photobioreactor is low cost, when compared with other bubble column reactors, mainly due to its sparger type.

The first part of this work was the determination of the photobioreactor characterization parameters. The highest rotation of the sparger was reached for the highest gas flow rates and for the minimum liquid volume tested. Concerning to gas hold up, the maximum values were registered for the highest gas speeds and for the minimum liquid level in the reactor. The obtained values are low. This can be due to the bubble size, which strongly depends on the sparger type used. The smaller the bubbles, the greater the gas hold up values. For mixing time, the trend was a declining mixing time with increasing gas flow rate. It was found that for higher flow rates the rotation of the sparger, although higher, makes no improvement in the mixing time. The  $k_{La}$  increases with increasing gas flow rate for all operating conditions. It was verified a tendency for higher  $k_{La}$  values in the case of the dynamic sparger that, although it might be within the measuring error, seems to indicate advantage of the sparger rotation in mass transfer.

The *Scenedesmus obliquus* cultivation was successful and lasted 14 days. The results of the daily follow-up of both cultures did not show evident differences between the photobioreactor with the static sparger and the one with the dynamic sparger.

Regarding the analysis of the composition in fatty acids of the samples obtained during the culture of *Scenedesmus*, it was verified that, besides containing low lipid content, the oil from microalgae from both photobioreactors did not have a suitable composition for biodiesel production.

Finally, it was used an industrial anodising sludge and was studied its capacity as coagulant of a microalgae broth, as a first step of biomass harvesting. It was verified some

flocculation capacity of the tested mud but the flocculation conditions are still to be optimized.

The development of closed photobioreactors with low cost and low maintenance is essential for the sustainable production of biofuels from autotrophic microalgal sources. However, there's still a gap between the design of a photobioreactor that on one hand is capable of supply all the microalgae growth requirements and on the other hand being cost effective from the perspective of construction and maintenance, increasing the economic viability of the process. It can be concluded that the photobioreactor studied is adequate for the growth of microalgae having the advantages of being a low cost reactor and enabling a high working volume (70 L). However the optimization of the experimental conditions is still needed, such as the optimum gas flow rate that allows an efficient oxygen stripping.

However, great efforts on R&D should be under taken until microalgal oil production becomes economically competitive and implemented on a large scale.

#### 5. References

- [1] J. C. Escobar, E. S. Lora, O. J. Venturini, E. E. Yáñez, E. F. Castillo, O. Almazan, Biofuels: Environment, technology and food security., Renewable and Sustainable Energy Reviews. 13 (2009) 1275–1287.
- [2] A. Perimenis, H. Walimwipi, S. Zinoviev, F. Müller-Langer, S. Miertus, Development of a decision support tool for the assessment of biofuels., Energy Policy. 39 (2011) 1782–1793.
- [3] L. Brennan, P. Owende, Biofuels from microalgae - A review of technologies for production, processing, and extractions of biofuels and co-products., Renewable and Sustainable Energy Reviews. 14 (2010) 557–577.
- [4] G. Huang, F. Chen, D. Wei, X. Zhang, G. Chen, Biodiesel production by microalgal biotechnology., Applied Energy. 87 (2010) 38–46.
- [5] T. M. Mata, A. a. Martins, N. S. Caetano, Microalgae for biodiesel production and other applications: A review., Renewable and Sustainable Energy Reviews. 14 (2010) 217–232.
- [6] O. Pulz, Photobioreactors: production systems for phototrophic microorganisms., Applied Microbiology and Biotechnology. 57 (2001) 287–293.
- [7] N. Kantarci, F. Borak, K. Ulgen, Bubble column reactors., Process Biochemistry 40 (2005) 2263-2283.
- [8] M. Bouaifi, G. Hebrard, D. Bastoul, M. Roustan, A comparative study of gas hold-up, bubble size, interfacial area and mass transfer coefficients in stirred gas-liquid reactors and bubble columns., Chemical Engineering and Processing 40 (2001) 97-111.
- [9] R. Krishna, J. M. van Baten, Mass transfer in bubble column, Catalysis Today 79-80 (2003) 67-75.
- [10] A. S. Mirón, M. C. C. García, F. G. Camacho, E. M. Grima, and Y. Chisti, Mixing in Bubble Column and Airlift reactors, IChemE, Part A, October 2004 1367-1374.
- [11] A. P. Carvalho, L. a Meireles, F. X. Malcata, Microalgal reactors: a review of enclosed system designs and performances., Biotechnology progress. 22 (2006) 1490–506.

- [12] E. M. Grima, E.-H. Belarbi, F. G. A. Fernández, A. R. Medina, Y. Chisti, Recovery of microalgal biomass and metabolites: process options and economics., *Biotechnology Advances* 20 (2003) 491-515.
- [13] E. W. Becker, Measurement of algal growth, in: S. J. Baddiley, N. H. Carey, I. J. Higgings, W. G. Potter (Ed.), *Microalgae, Biotechnology & Microbiology*, Cambridge University Press, 1994: pp. 56-62.
- [14] American Public Health Association, America Water Works Association, and Water Environment Federation, Standard methods for the examination of water and wastewater. NO<sub>3</sub><sup>-</sup> ultraviolet spectrophotometric screening method. 20th edition. APHA, AWWA, WEF, Washintong, DC, 1998: pp. 4-115.
- [15] G. Lepage, C. C. Roy, Direct transesterification of all classes of lipids in a one-step reaction., *Journal of Lipids Research*. 27 (1986) 114–120.
- [16] European Standard EN 14214, Automotive fuels – fatty acid methyl esters (FAME) for diesel engines – requirements and test methods., AFNOR, Sain-Denis, 2004. Available at: <http://www.novaol.it/novaol/export/sites/default/allegati/EN14214.pdf>.
- [17] M. J. Ramos, C. M. Fernández, A. Casas, L. Rodríguez, A. Pérez, Influence of fatty acid composition of raw materials on biodiesel properties., *Bioresource Technology*. 100 (2009) 261–268.