INTEGRATED MASTER IN BIOENGINEERING

EVALUATION OF ELECTROFLOCCULATION FOR HARVESTING DUNALIELLA SALINA USING NON SACRIFICIAL ELECTRODES

MASTER THESIS

of

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Developed within the discipline of Dissertation

Conducted at

a4f
algae for future

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July 2016
Dedicated to my family,

for their support
and unconditional love.
Acknowledgments

I would like to thank Dr. Vítor Verdelho for the opportunity to work in A4F. I would also like to thank Dr. Luís Costa and Dr. Pedro Fonseca, from A4F, for the guidance, support and valuable expert advice during this Project. I am also thankful to Joana Lapa for the encouragement throughout this project and friendship.

I would like to express my sincere appreciation to Professor Domingos Barbosa, from FEUP, for the ineffable aid during my thesis work and for the critical reading of this report, its structure and content.

I am also thankful for the undeniable support of the Department faculty members, Carla, Sílvia, Paula and Luís Carlos for their patience and kindness.

I would like to express my gratitude to my colleagues with whom I had the pleasure of sharing the past 5 years at the Faculty of Engineering of the University of Porto.

To my friends Ana Isabel and Débora, thank you for your friendship and encouragement.

To my supportive and ever present friends, Ivo and Mauro, with whom I had shared my best experience during the course, an Erasmus internship; I would like to thank you for all. You will be always in my heart.

To my boyfriend Octávio, a special thanks for sharing these years with me, for the patience in the most difficult moments and the endless love.

This work was financially supported by: Project POCI-01-0145-FEDER-006939 (Laboratory for Process Engineering, Environment, Biotechnology and Energy – LEPABE funded by FEDER funds through COMPETE2020 - Programa Operacional Competitividade e Internacionalização (POCI) – and by national funds through FCT - Fundação para a Ciência e a Tecnologia.
Abstract

Microalgae harvesting process accounts for 20 - 30% of the total cost associated with microalgae production and processing. Because of that, their commercial large scale cultivation is currently limited to the production of high value metabolites such as carotenoids. One such example is the microalgae Dunaliella salina large accumulation of β-carotene in response to various stress conditions. There are companies in Portugal interested in harnessing the potential of Dunaliella salina. One such company is A4F - Algae for future, with which this work has been developed.

In this work it was studied the electroflocculation method for harvesting orange phase Dunaliella salina from the culture medium by using graphite electrodes, which reduces operating costs and environmental contamination due to insignificant weight loss thereof during the assays. The effect of several parameters such as the electrode distance, electroflocculation time, stirring speed, current intensity and electrical conductivity on harvesting efficiency and power consumption was studied. The maximum harvesting efficiency achieved by this method was 71.1% with an energy consumption of 0.63 kWh/m³, using an electrode distance of 1 cm, an electroflocculation time of 6 min, a stirring speed of 400 rpm and a current intensity of 0.7 A. The results on the influence of the electrical conductivity reveal that the energy consumption is lower for saltwater than freshwater, which is an advantage for Dunaliella salina harvesting. Cell viability was studied with FDA and was used as a complementary method. It was found that the viability decreases with the increase of both current intensity and stirring speed, possibly due to the formation of a mucous layer surrounding the cell. For the optimized parameters cell viability was 21.2%. Cell viability shall be used as a complementary methodology when electroflocculation parameters will be setting. However, cell viability methodology needs to be improved.

The results achieved in this study reveal that electroflocculation has a high potential and is a promising harvesting technique, due to the absence of chemicals in the culture medium and low maintenance costs. However, further tests are needed to better understand the influence of each parameter in the membrane cell.

Keywords: Dunaliella salina, harvesting, electroflocculation, graphite electrodes, cell viability.
Resumo

O processo de colheita das microalgas corresponde a cerca de 20-30% dos custos totais associados à sua produção e ao processamento. Por isso, a cultivação em larga escala está atualmente limitada à produção de metabolitos de valor acrescentado, como é o caso dos carotenoides. Um exemplo é a microalga *Dunaliella salina*, que é capaz de acumular grandes quantidades de β-caroteno em resposta a várias condições de stress. Existem empresas em Portugal que estão interessadas em explorar o potencial da *Dunaliella salina*, como é o caso da A4F – Algae for future, com a qual este trabalho foi desenvolvido.

Neste trabalho foi estudada a eletrofloculação como método de concentração da *Dunaliella salina*, na fase laranja, e usaram-se elétrodos de grafite, uma vez que reduzem os custos de operação e a contaminação ambiental por não sofrerem perda de massa ao longo dos ensaios. Ao longo deste trabalho estudaram-se os efeitos de alguns parâmetros da eletrofloculação, como a distância entre os elétrodos, o tempo de eletrofloculação, a velocidade de agitação, a intensidade de corrente e a condutividade elétrica, na eficiência de remoção e no consumo de energia. A eficiência de remoção máxima atingida por este método foi de 71.1% com um consumo de energia de 0.63 kWh/m³. Para tal, os elétrodos foram dispostos a 1 cm de distância, o processo de eletrofloculação durou 6 minutos e foram aplicadas uma velocidade de agitação de 400 rpm e uma intensidade de corrente de 0.7 A. O estudo da influência da condutividade elétrica na eletrofloculação revelou que o consumo de energia é menor quando a eletrofloculação é aplicada a águas salinas em vez de águas com baixa concentração de sal, o que revela ser uma vantagem na colheita da *Dunaliella salina*. A viabilidade celular foi estudada por microscopia com recurso ao fluorocromo FDA e foi usada como um método complementar. Observou-se que a viabilidade celular diminuiu quer com o aumento da corrente quer com o aumento da velocidade de agitação, devido à formação de uma camada de muco ao redor das células. Para os parâmetros otimizados observou-se uma viabilidade celular de 21.2%. Assim, a viabilidade celular deverá ser usada com uma metodologia complementar aquando da otimização dos parâmetros da eletrofloculação. No entanto, a metodologia utilizada no estudo da viabilidade celular precisa de ser melhorada.

Os resultados obtidos neste estudo revelam que a eletrofloculação tem um grande potencial e é uma técnica de colheita promissora devido à ausência de químicos no meio de cultura e aos baixos custos de manutenção. No entanto, serão necessários mais testes para perceber melhor a influência de cada parâmetro da eletrofloculação na membrana celular.

*Palavras-chave:* *Dunaliella salina*, colheita, eletrofloculação, elétrodos de grafite, viabilidade celular.
Declaration

It is declared that this work is original and that all non-original contributions have been properly referenced with the identification of the source.

______________________________________________, ___of ______ of 2016

Ana Luísa Gonçalves Teixeira
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<thead>
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<th>Symbol</th>
<th>Definition</th>
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<tbody>
<tr>
<td>C</td>
<td>Concentration, g/L</td>
</tr>
<tr>
<td>I</td>
<td>Electrical current, A</td>
</tr>
<tr>
<td>M</td>
<td>molar</td>
</tr>
<tr>
<td>P</td>
<td>Power, kWh/m³</td>
</tr>
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<td>S</td>
<td>Surface area, m²</td>
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<td>T</td>
<td>Temperature</td>
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<td>V</td>
<td>Volume, L</td>
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<tr>
<td>w/v</td>
<td>Microalgae dry mass/volume</td>
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Abbreviations and acronyms

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
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<tr>
<td>Car</td>
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<td>Chl</td>
<td>Chlorophylls</td>
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<td>CO₂</td>
<td>Carbon dioxide</td>
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<tr>
<td>e.g.</td>
<td>For example</td>
</tr>
<tr>
<td>EC</td>
<td>Electrical conductivity (S/m)</td>
</tr>
<tr>
<td>EF</td>
<td>Electroflocculation</td>
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<tr>
<td>FDA</td>
<td>Fluorescein diacetate</td>
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<tr>
<td>HE</td>
<td>Harvesting efficiency</td>
</tr>
<tr>
<td>NaCl</td>
<td>Sodium Chloride</td>
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<tr>
<td>OD</td>
<td>Optical density (680 nm)</td>
</tr>
<tr>
<td>Oda</td>
<td>Optical density after electroflocculation (680 nm)</td>
</tr>
<tr>
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<tr>
<td>PI</td>
<td>Propidium iodide</td>
</tr>
<tr>
<td>rpm</td>
<td>Rotations per minute</td>
</tr>
<tr>
<td>TSS</td>
<td>Total suspended solids</td>
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Chapter 1: Introduction

1.1 Background and Project Presentation

Microalgae are among the fastest growing autotrophs on the earth which are able to convert carbon dioxide and water in valuable chemicals using sunlight and a few nutrients. Furthermore, microalgae have a worldwide distribution and are well-adapted to survive under a large spectrum of environmental conditions, including heat, cold, salinity, photo-oxidation and anaerobiosis (Tandeau de Marsac & Houmard 1993).

Microalgae combine typical properties of higher plants (e.g., efficient oxygenic photosynthesis and simple nutritional requirements) with properties of microorganisms (e.g., fast growth rates and ability to accumulate or secrete primary and secondary metabolites). This combination represents the great advantage of microalgae biotechnology (Guedes et al. 2011).

Microalgae are a good source of nutraceuticals, that can be defined as "a food (or part of a food) that provides medical or health benefits, including the prevention and/or treatment of a disease” (DeFelice 2012). Some nutraceuticals commonly found in microalgae include high quality proteins, essential amino acids, antioxidants, vitamins, minerals, essential fatty acids and others (Fallis 2013). Increasing consumer health awareness is leading to a growing demand for them. Microalgae are also a good source of pharmaceuticals that are used to diagnose, cure or prevent diseases. The major classes of pharmaceuticals include antimicrobial, antiviral, anticancer and anti-inflammatory agents (Chidambara Murthy 2005).

The key factor for the microalgae economic feasibility is the possibility of operating in large scale. However, microalgae cultures need to be concentrated for further dewatering of the biomass or extraction of valuable products, because they exist as a dilute suspension containing, typically, 0.5 - 5.0 g of dried biomass per liter. Furthermore, harvesting process accounts for 20 - 30% of the total costs associated with microalgae production and processing (Al Hattab et al. 2015; Christenson & Sims 2011; Grima et al. 2003). Because of that, commercial large scale cultivation of microalgae is currently limited to the production of high value metabolites such as carotenoids, omega-3 fatty acids and lipids that find application in health food, cosmetics, food supplements, pharmaceuticals and biofuel production (Fallis 2013; DeFelice 2012)
The large number of microalgae species is a unique reservoir of biodiversity, which supports potential commercial use of many new products from species able to give high yield at low production cost.

One such example is the unicellular microalga *Dunaliella salina* which was a large accumulation of β-carotene in response to various stress conditions (Oren 2005). As *Dunaliella salina* is found in natural salt pans and salt lakes, the harvesting method is an essential step for the economical production of natural β-carotene from microalgae.

There are companies in Portugal interested in harnessing the potential of *Dunaliella salina*, since there are several natural salt pans along the country where this microalga is produced. One such company is A4F – Algae for future, with which this work was developed.

### 1.2 The company A4F - Algae for future, S.A.

A4F – Algae for future, is a Portuguese bioengineering company with more than 15 years of accumulated expertise in research, development, industrial production and marketing of microalgae and microalgae applications. A4F works with different microalgae species, different production solutions, different harvesting methods and downstream technologies. This company believes that microalgae have multiple high value applications, and their production and industrial treatment can, in itself, ensure economic and environmental benefits (A4F Algae For future n.d.).

The increasing interest in β-carotene and β-carotene based products creates a demand for novel production and harvesting methods. In Portugal, for example, there are several salt pans and salt lakes where β-carotene is naturally produced by *Dunaliella salina*. This microalga is the starting point in the food chain of the ecosystem of salt pans, because it feeds *Artemia salina*, a macro-invertebrate that is the basis of the food chain of various aquatic birds (Necton n.d.). In order to economically extract the β-carotene from these microalgae a harvesting method suitable to the various productions sites is needed.

### 1.3 Main objectives

The main objectives for this project are:

i) Development of an economically and environmentally sustainable method for harvesting *Dunaliella salina* blooms in the orange phase (high carotenoids concentration) by electroflocculation.
Evaluation of electroflocculation for harvesting *Dunaliella salina* using non sacrificial electrodes.

**ii)** Evaluation of electroflocculation for harvesting *Dunaliella salina* using non sacrificial electrodes.

**iii)** Development of a methodology to evaluate the effect of electroflocculation on cell viability.

### 1.4 Thesis organization

The thesis is organized in six chapters. The present one, Chapter 1, introduces the motivations and main objectives of this work.

Chapter 2 provides a literature review focusing on the available technologies for *Dunaliella salina* harvesting and their main advantages and limitations. It also includes a review of the use of *Dunaliella salina* for the production of β-carotene.

The materials and methods used to study the influence of the main parameters of the electroflocculation process, namely, electrode distance, electroflocculation time, stirring speed, current intensity and electrical conductivity, on the harvesting efficiency and power consumption are described in Chapter 3. The study of *Dunaliella salina* viability is also described in this chapter.

In Chapter 4, the results and their discussion are presented, following the same pattern of the previous chapter.

The conclusions withdrawn from this work are presented in Chapter 5, and some suggestions for future work and a final appreciation of the work developed in this thesis is given in Chapter 6.
Introduction
Chapter 2: Literature review

2.1 Dunaliella salina

*Dunaliella salina* is a unicellular green alga, which belongs to the phylum of *Chlorophyta*, order *Volvocales*, family *Polyblepharidaceae* and the genus *Dunaliella* found in environments with high salt concentration, that produces a distinct orange color often found in salt pans (Oren 2005; Ramos *et al.* 2011). The first scientific records of the brine algae *Dunaliella* date 1838 when Dunal, a French engineer, showed interest on the pink-red coloration of the salt fields in the Mediterranean. He observed that the microorganisms were biflagellate cells, and identified them as *Haematococcus*. However, *Dunaliella* was not named until 1905, when Teodoresco noted that, unlike *Haematococcus*, they lacked cell wall. Therefore, he gave them the name *Dunaliella* in honor of the scientist Dunal (Oren 2005; Borowitzka 1998).

Till now there were observed 29 species of *Dunaliella*, but this study will focus on *Dunaliella salina* because of its known high β-carotene accumulation. The morphological characteristic of *Dunaliella*, in contrast to other members of *Chlorophyta*, is that it lacks a rigid polysaccharide cell wall having, instead, a mucus surface coat.

*Dunaliella salina* is able to tolerate varying sodium chloride (NaCl) concentrations, ranging from 0.05 M to approximately 5.5 M, and temperatures ranging from 0 °C up to 40 °C. However, optimal growth is obtained at 2 M NaCl and 32 °C (Hosseini Tafreshi & Shariati 2009). Thus, *Dunaliella salina* is a hyper-halotolerant organism, which accumulates glycerol to balance osmotic pressure, enclosed by an elastic membrane, which is responsible for its rapid change in shape and response to osmotic variations (Borowitzka & Siva 2007; Shariati & Reza 2011; Vanita 2007). *Dunaliella salina* is also adapted to solar radiation using β-carotene for protection against ionizing energy (Schlipalius 1991).

The cell size in *Dunaliella* species can vary from 5 to 25 µm in length and from 3 to 13 µm in width. The cell contains one large cup shaped chloroplast, which occupies half of the cell volume, which accumulates large quantities of β-carotene within oily globules in the inter-thylakoid spaces, responsible for the cells orange rather than green color (Borowitzka & Borowitzka 1990; Hosseini Tafreshi & Shariati 2009).

As already mentioned, *Dunaliella salina* is a biflagellate alga with two long flagella equal in length. Under extreme salt concentration, greater than 4 M, *Dunaliella salina* loses its flagella, forming a dehydration resistant cyst (Seckbach 1999).
The major growth requirements of *Dunaliella salina* are nutrients, light and temperature. Nutrients requirements for *Dunaliella salina* include nitrates, phosphates and sulphates. Light induces photosynthesis capacity and it plays a vital role in *Dunaliella salina* growth as well as in carotenogenesis, where massive accumulation of β-carotene occurs. The influence of temperature on growth is associated with various metabolic processes like photosynthesis, membrane permeability, respiration, and others (Chidambara Murthy 2005).

### 2.2 Carotenoids

All photosynthetic organisms contain organic pigments for capturing light energy. There are three major classes of pigments: chlorophylls, carotenoids and phycobilins. The chlorophylls (green pigments) and carotenoids (yellow or orange pigments) are lipophilic, while phycobilins are hydrophilic.

Due to its ability to produce orange pigmentation, β-carotene, a natural food coloring, is applied to a range of food and beverages products to improve their appearance, is also highly demanded for as well as to cosmetic products, due to its anti-oxidant effect, and as an additive in human and animal nutrition as source of pro-vitamin A (retinol) (Martinez *et al.* 1995; Shariati & Reza 2011). Although β-carotene can be produced by chemical synthesis with high purity and at low cost, the chemical synthesis produces mixtures of stereo isomers that are not appropriate for human consumption because of their undesired side effects (Ben-Amotz & Yishai Levy 1996). Therefore, it would be a better and healthy choice to produce natural β-carotene.

Under favorable growth conditions, vegetative cells of the green alga *Dunaliella* can synthesize around 0.4% of its dry alga mass as β-carotene, which is necessary for its photosynthesis. However, when vegetative green cells are exposed to environmental stress conditions, some species of *Dunaliella* synthesize and accumulate β-carotene up to 14% of its dry alga mass. *Dunaliella salina* produces mainly two β-carotene stereoisomers: *all-trans* and 9-cis (Seckbach 1999).

In Figure 1 it is shown the carotenogenic environmental conditions for *Dunaliella salina*. When it grows in natural open-ponds, *Dunaliella salina* has an annual cycle. In autumn – winter, there is greater rainfall (and consequent higher dilution of salt), temperatures are lower and there is no nutrient limitation, which leads to a high growth of *Dunaliella salina*, forming blooms (A). In the spring-summer, there is less rainfall, temperatures are higher, there is more evaporation, more incident sunlight and there is a limitation of nutrients because of their precipitation. These factors have an additive or synergistic effect on carotenogenesis and
Dunaliella salina become orange (Farhat et al. 2011; Kitto & Reginald 2011; Varela et al. 2015). When the pond has reached an appropriate quantity of Dunaliella salina biomass, the culture may be harvested. For large-scale outdoor algal cultures, productivities of Dunaliella salina of 30–40 g(dry mass).m\(^{-2}\).day\(^{-1}\) seem to represent the present achievable limit (Borowitzka 1990; Ralefala 2011).

![Carotenogenic environmental conditions for Dunaliella salina](image)

**Figure 1.** Carotenogenic environmental conditions for Dunaliella salina (Varela et al. 2015).

Although a fraction of β-carotene participates in the photosynthesis, during carotenogenesis additional β-carotene may be over-accumulated in lipid globules in the chloroplast. As a consequence, cells not only upregulate the level of cellular β-carotene but they also change the location of β-carotene deposition. Therefore, globules formation, in which β-carotene is deposited during carotenogenesis, also requires lipids to be produced. Thua, it is expected that the mechanisms of β-carotene accumulation and lipid production in cells are related (Varela et al. 2015).

Currently, it is still uncertain how the various stress conditions are being sensed by Dunaliella salina and what mechanisms are involved in signal transduction that ultimately results in over-accumulation of β-carotene.
2.2.1 Global market of β-carotene

The commercial production of β-carotene from *Dunaliella salina* is a well-exploited industry. Nowadays, Australia produces over 80% of the world’s natural β-carotene. The first companies to attempt the massive culture of *Dunaliella salina* were Western Biotechnology and Betatene Ltd (which was acquired by Henkel-Cognis and after, in 2010, was acquired by BASF) (Borowitzka 1990; Fallis 2013; Hosseini Tafreshi & Shariati 2009). These companies grow the algae in very large (3,000 - 10,000 m²) and shallow (20 - 30 cm deep) ponds constructed either on the bed of a hyper saline coastal lagoon or formed by artificially expanding a lagoon. There are no mixing devices at the ponds. The only mixing is by wind and thermal action. Although this form of extensive cultivation results in lower cell densities compared to a well mixed system, it is economically more attractive as long as an effective and cheap harvesting process can be used to extract the algae from the extremely large volumes of brines (Borowitzka 1990; Schlipalius 1991).

β-carotene can be sold in various formulations, as pure beta-carotene or mixed carotenoids, as a nutritional supplement, and natural food coloring used for the production of sweets, ice creams, milk, beverages and processed foods. Dried *Dunaliella salina* powder is also sold as a feed additive for aquaculture to pigment crustaceans such as prawns.

The global carotenoids market value was 1.3 billion € in 2014. β-carotene is the most sold carotenoid globally, accounting for 231 million € in 2010 and will be worth 295 million € in 2018, with an annual growth rate of 3.1% (BCC research 2015).

The price of natural β-carotene formulations can vary from 269 - 2,692 € per kg. Production costs of *Dunaliella salina* should not exceed 45 – 90 € per kg, to account for losses at each processing step, capital expenses, marketing, packaging and distributions costs (Borowitzka & Borowitzka 1989; Richmond & Ben-Amotz 2004).

2.3 *Dunaliella salina* harvesting

After culturing the microalgae, the biomass needs to be separated from the culture medium for further processing, an operation that is known as “harvesting”. This step is one of the most difficult ones due to the low microalgae concentration, which means that large amounts of water have to be processed.

The methodology that is generally applied to most microalgae, which possess rigid cell wall, is a two-step concentration: thickening followed by dewatering. In the first step, the microalgae suspension is thickened to a slurry of about 2 – 7% of total suspended solids, TSS,
Literature review

Evaluation of electroflocculation for harvesting *Dunaliella salina* using non sacrificial electrodes

This step works as a pre-concentration treatment and contributes to a significant volume reduction, which is important to reduce downstream processing costs. In the second step, the slurry is dewatered to a cake with 15 – 25% TSS (concentration factor of 2 – 10). This step needs more energy than thickening (Barros *et al.* 2015; Brennan & Owende 2010; Vandamme *et al.* 2013).

Flocculation, flotation, electrically based methods or gravity sedimentation are the most frequently used methods in the first step (thickening) where filtration and centrifugation are the most used in the second step (dewatering) (Barros *et al.* 2015). This methodology is the key to obtain thick algal slurry from the initial suspension.

The selection of the appropriate harvesting method depends on the initial culture medium and the final product characteristics. Thus, it is necessary to take into account the specific features of *Dunaliella salina*, as: i) lack of cell wall; ii) an outer cell layer which produces mucus; iii) elasticity of the cell membrane; iv) high extracellular salinity; v) small size (5 ~ 25 µm); vi) negative surface charge (from -7.5 up to -40 mV); vii) low microalgae biomass concentration (0.5 ~ 5 g/L) and viii) high electrical stability (Seckbach 1999). These factors exclude many routine harvesting methods used frequently in microalgae biotechnology.

A range of commercial harvesting techniques are currently available that include centrifugation, sedimentation, flotation, flocculation, filtration and electrical based methods as electroflocculation. An ideal harvesting process should allow the achievement of high biomass concentrations, while requiring moderate costs of energy, operation and maintenance. There are numerous patents relating to harvesting methods that were used over the years and others, more recent, which are still under study. Some of these patents relating to the microalgae harvesting methods are described in Table 15 in Appendix 1.

In Table 1 are compared existing and in development harvesting technologies from the technical and economic point of view. It can be seen that centrifugation, chemical coagulation/flocculation, gravity sedimentation, air flotation, dead-end filtration and cross flow filtration are not good methods to harvesting *Dunaliella salina* due to their high energy/maintenance costs, low concentration and the contamination of the biomass and culture medium.
Table 1. Comparison of harvesting methods for microalgae (Al Hattab et al. 2015; Barros et al. 2015; Brennan & Owende 2010; Curtain & Snook 1985; Pirwitz et al. 2015; Uduman et al. 2010).

<table>
<thead>
<tr>
<th>Harvesting method</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Centrifugation</td>
<td>• High solids concentrations</td>
<td>• High capital and operating costs</td>
</tr>
<tr>
<td></td>
<td>• Fast method</td>
<td>• High energy costs</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Possibility of cell damage due to high shear forces</td>
</tr>
<tr>
<td>Chemical coagulation/flocculation</td>
<td>• High yields</td>
<td>• Require high dosages of chemicals that may be expensive</td>
</tr>
<tr>
<td></td>
<td>• Simple and fast method</td>
<td>• Contamination of the culture medium</td>
</tr>
<tr>
<td></td>
<td>• No energy requirements</td>
<td></td>
</tr>
<tr>
<td>Gravity sedimentation</td>
<td>• Low energy requirement</td>
<td>• Time consuming</td>
</tr>
<tr>
<td></td>
<td>• Simple method</td>
<td>• Low solids concentration</td>
</tr>
<tr>
<td>Air flotation</td>
<td>• Fast method</td>
<td>• Oxidation of the caroteniferous material</td>
</tr>
<tr>
<td></td>
<td>• Feasible for large scale applications.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Continuous process</td>
<td></td>
</tr>
<tr>
<td>Dead-end filtration</td>
<td>• High recovery efficiency</td>
<td>• High energy costs</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Membrane fouling</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• High equipment/maintenance costs</td>
</tr>
<tr>
<td>Cross-flow filtration</td>
<td>• Increases the lifespan of the filter</td>
<td>• Poor dewatering rate</td>
</tr>
<tr>
<td></td>
<td>• Continuous process</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Flexible method</td>
<td></td>
</tr>
<tr>
<td>Electrofloculation with sacrificial electrodes</td>
<td>• High yields</td>
<td>• Not a continuous process</td>
</tr>
<tr>
<td></td>
<td>• Compact equipment</td>
<td>• High maintenance costs (depletion of the cathode)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Contamination of the product and the culture medium</td>
</tr>
<tr>
<td>Electrofloculation with non sacrificial electrodes</td>
<td>• Absence of product and medium contamination</td>
<td>• Under evaluation</td>
</tr>
<tr>
<td></td>
<td>• High yields</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Compact equipment</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Continuous process</td>
<td></td>
</tr>
</tbody>
</table>

A comparison of the energy consumption with various microalgae dewatering techniques is made in Table 2.
Table 2. Power consumption comparison for various dewatering techniques.

<table>
<thead>
<tr>
<th>Dewatering method</th>
<th>Power consumption (P) (kWh/m³)</th>
<th>Suspended solids (SS) in concentrate</th>
<th>Ratio P/SS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Centrifugation¹</td>
<td>8.00</td>
<td>22.00</td>
<td>0.36</td>
</tr>
<tr>
<td>Tangential flow filtration²</td>
<td>0.30 - 2.06</td>
<td>8.88</td>
<td>0.04</td>
</tr>
<tr>
<td>Vacuum filtration³</td>
<td>5.90</td>
<td>18.00</td>
<td>0.33</td>
</tr>
<tr>
<td>Pressure filtration⁴</td>
<td>0.88</td>
<td>22.00 – 27.00</td>
<td>0.03</td>
</tr>
<tr>
<td>Polymer flocculation²</td>
<td>14.81</td>
<td>15.00</td>
<td>0.99</td>
</tr>
<tr>
<td>Dissolved air flotation⁴</td>
<td>7.60</td>
<td>4.50</td>
<td>1.69</td>
</tr>
<tr>
<td>Electroflocculation⁵</td>
<td>0.33</td>
<td>Not determined</td>
<td>-</td>
</tr>
</tbody>
</table>

¹ Scenedesmus, C. proboscideum, (Grima et al. 2003; Poelman et al. 1996).
³ C. p roboscideum, (Grima et al. 2003; Poelman et al. 1996).
⁴ Chlorella and Scenedesmus, (Wiley et al. 2009).
⁵ Dunaliella salina (green phase), (Zenouzi et al. 2013).

Although current values of power consumption in the microalgae industry can be significantly lower due to technological development and operation parameter optimization, for the particular cases presented in the previous table, it can be seen that centrifugation consumes 8.00 kWh/m³ a significant amount of energy, to concentrate the microalgae culture.

Comparing the different filtration methods, it was found that pressure filtration appeared to be more energy efficient, consuming 0.88 kWh/m³ in order to concentrate the microalgae culture. The vacuum filtration appeared to be the least energy efficient of the filtration techniques, consuming 5.90 kWh/m³ to concentrate the microalgae culture. Tangential flow filtration had an intermediary energy consumption of approximately 2.06 kWh/m³ to concentrate the microalgae culture.

Polymer flocculation appears to be the least energy efficient, which may be explained by the need to mix the suspension to be treated.

The energy consumption required by electroflocculation method is lower than the energy that is required by other methods described above, which makes the studied electroflocculation method a promising alternative to be used in industrial applications.

Therefore, and after considering the various harvesting methods, it was concluded that electroflocculation seems to be a promising harvesting method for the microalgae Dunaliella salina to be competitive with the production of synthetic β-carotene.

On electroflocculation method can be used both sacrificial and non sacrificial electrodes. When sacrificial electrodes are used in the electroflocculation process, the flocculating agents are ions which precipitated from the sacrificial anode, since they are made of an inactive metal (non sacrificial). The metal ions collide with suspended particles in the electrolyte,
adsorbing onto their surface, and the hydrogen gas adheres to the microalgal flocs and carry them to the surface, where the layer formed can be removed by skimming (Uduman et al. 2010). The best active anode materials are iron and aluminum as they give bivalent and trivalent ions which have a higher ability to be adsorbed due to their higher charge density (Syversen 1995). However, these ions cause a contamination of the culture medium and of the recovered biomass, which leads to extra costs and safety issues.

In order to reduce the use of chemicals to aid microalgal recovery, it will be studied in this work an electrolytic method, electroflocculation, with non sacrificial electrodes. Table 3 shows the SWOT analysis to the harvesting processes that will be studied, i.e. electroflocculation. In the present SWOT analysis, directed to A4F company, there are identified the strengths, weaknesses, opportunities and threats for *Dunaliella salina* electroflocculation.

**Table 3.** Electroflocculation SWOT analyses, adapted from (Makonin et al. 2016; Uduman et al. 2010).

<table>
<thead>
<tr>
<th>Strengths</th>
<th>Weakness</th>
</tr>
</thead>
<tbody>
<tr>
<td>• No chemicals are required</td>
<td>• Limited experience/knowledge on large scale application</td>
</tr>
<tr>
<td>• Reduced operation cost</td>
<td>• Limited knowledge about the effect of electroflocculation on microalgal surface</td>
</tr>
<tr>
<td>• Low maintenance cost</td>
<td>• Microalgae phase dependent (membrane properties)</td>
</tr>
<tr>
<td>• Versatile</td>
<td>• Difficult entry into the market due to strong competition from world established companies</td>
</tr>
<tr>
<td>• Betting on innovation and sustainability</td>
<td></td>
</tr>
<tr>
<td>• Strong A4F presence of algae market</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Opportunities</th>
<th>Threats</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Explore new markets</td>
<td>• Scale-up from the lab to large scale</td>
</tr>
<tr>
<td>• Highest demand of β-carotenes based products</td>
<td>• Low knowledge of its technical and economic viability</td>
</tr>
<tr>
<td>• More useful in marine water than fresh water</td>
<td>• Producers with a established distribution network</td>
</tr>
<tr>
<td>• Compete with established companies due to the lower operation cost</td>
<td></td>
</tr>
</tbody>
</table>
2.4 Electroflocculation

Electroflocculation is based on the principle of the movement of electrically charged particles in an electric field. The negative surface charge of *Dunaliella salina* causes their movement towards the anode, which has a positive charge, during this procedure. When non sacrificial electrodes are used, and in close proximity to the anode, the charges are neutralized and *Dunaliella salina* flocs are formed and float to the surface where they can be skimmed off (Misra *et al.* 2015). This can be seen in Figure 2, which shows a schematic diagram of an electroflocculation unit.

![Figure 2](image-url)

**Figure 2.** Schematic diagram of an electroflocculation unit during electroflocculation (a) and after electroflocculation procedure (b) (adapted from Vispute 2014).

There is a difference in the electrolys products obtained by dilute or concentrate sodium chloride (NaCl) solutions.

In a dilute solution of NaCl (<5% NaCl) there are four different types of ions in solution: Na⁺ (aq), Cl⁻ (aq), H⁺ (aq) and OH⁻ (aq).

The H⁺ and Na⁺ ions are attracted to the cathode, which has a negative charge. There, H⁺ ions gain electrons, because hydrogen ions have lower relative stability and higher tendency to discharge (lower oxidation-reduction potential) than the Na⁺ ions, to form H₂ (g), which bubbles off the solution, while Na⁺ ions remain in solution.

\[ 2H^+(aq) + 2e^- \rightarrow H_2 (g) \]  \[ (1) \]

The OH⁻ and Cl⁻ ions are attracted to the anode. There, OH⁻ ions give up electrons to the anode, due to their higher tendency to discharge (lower oxidation-reduction potential than Cl⁻), to form water and oxygen gas while Cl⁻ ions remain in solution.
The overall reaction is:

$$4\text{OH}^- (aq) \rightarrow 2\text{H}_2\text{O} (l) + \text{O}_2 (g) + 4\text{e}^-$$  \hspace{1cm} (2)

The overall reaction shows that $\text{O}_2$ and $\text{H}_2$ are produced in the proportion of 1:2, and therefore the electrolysis of dilute sodium chloride solution is equivalent to the electrolysis of water. The pH of the solution remains the same after electrolysis.

When NaCl is present in high dosages there is the possibility of reduction and oxidation of both $\text{H}_2\text{O}$ and NaCl. In this case, the reaction that has the lower reduction-oxidation potential is the most likely to occur (Garcia 2002; Ramalho 2008).

In a concentrated solution of NaCl (>5% NaCl), Cl$^-$ have lower relative stability and higher tendency to discharge than OH$^-$ due to their high concentration in solution (L. R. Czarnetzki & Janssen 1992; The Nakhleh Group n.d.). Consequently, Cl$^-$ ions are discharged at the anode as chlorine gas (Cl$_2$), while OH$^-$ ions remain in solution. At the cathode, it is still produced $\text{H}_2$ since Na$^+$ continues more stable (high oxidation-reduction potential) even in high concentrations (L. R. Czarnetzki & Janssen 1992; The Nakhleh Group n.d.). In this case, Cl$_2$ and H$_2$ are produced in the proportion of 1:1. The Na$^+$ and OH$^-$ ions in solution can react and form NaOH as a sub-product of electrolysis. For concentrated solutions there may also occur the production of a stronger base, sodium hypochlorite (NaClO), where NaOH and Cl$_2$ are combined, leading to the increase of solution pH during the electrolysis.

The main reactions that occurs when a concentrate solution of NaCl is used are (L. R. Czarnetzki & Janssen 1992):

$$2\text{NaCl} (aq) \rightarrow 2\text{Na}^+ + 2\text{Cl}^-$$  \hspace{1cm} (4)

$$2\text{H}_2\text{O} \rightarrow 2\text{H}^+ + 2\text{OH}^-$$  \hspace{1cm} (5)

Anode:

$$2\text{Cl}^- \rightarrow \text{Cl}_2 (g) + 2\text{e}^-$$  \hspace{1cm} (6)

Cathode:

$$2\text{H}^+ \rightarrow \text{H}_2 (g)$$  \hspace{1cm} (7)

Global equation:

$$2\text{NaCl} + 2\text{H}_2\text{O} \rightarrow \text{H}_2 + \text{Cl}_2 + 2\text{Na}^+ + 2\text{OH}^-$$  \hspace{1cm} (8)

and,

$$2\text{NaOH} + \text{Cl}_2 \rightarrow \text{NaCl} + \text{NaClO} + \text{H}_2\text{O}$$  \hspace{1cm} (9)
One of the most common problems in the electroflocculation process of concentrate solutions of NaCl is the fouling of the cathode. This phenomenon occurs due to the increasing pH of the medium that causes the precipitation of carbonates and hydroxides of calcium and magnesium layers on the cathode. These two ions exist in the culture medium as nutrients for microalgae growth. Figure 3 shows the cathode and the anode after some electroflocculation assays.

Figure 3. Illustration of the cathode fouling (electrode on the left) and the absence of fouling in the anode (electrode on the right).

The increasing thickness of the fouling layer in the cathode reduces, gradually, the efficiency of the process and increases the resistance to the current (Hsu et al. 2015; Vandamme et al. 2011; Wijesekara et al. 2005). There are several options available to clean or prevent cathode fouling. Mechanical cleaning can be used, however, for continuous processes this is not a good option. The addition of inhibiting agents on electrode surface could prevent cathode fouling but it can lead to medium contamination and increase the harvesting cost. Thus, changing the polarity of the electrodes can be one of the most effective ways to solve this problem in continuous electroflocculation process (Rajic et al. 2014; Uduman et al. 2010; Syversen 1995).

Electroflocculation is a novel approach for microalgae harvesting (pre-concentration) that may benefit from the application of non sacrificial electrodes, an option that has not been thoroughly investigated. To better understand this process, it is important to study the factors that affect it, such as: time, current intensity, voltage, electrode material, electrodes distance and surface area, stirring speed, pH and salinity of the solution (Xiong et al. 2015).

**Current, Voltage and Time**

Current, voltage and time are the three most important factors that determine the harvesting efficiency achieved and the cost associated to the process of electroflocculation.
It is important to determine the optimum electroflocculation time of *Dunaliella salina* culture that allows achieving high harvesting efficiency preventing damage of the cells and reduction of power required.

The electroflocculation power requirement, the main contribution to the operating costs, can be calculated using equation 10 (Sathe 2010).

\[
P = \frac{V \times I \times t}{1000 \times v}
\]

where, \( P \) is the power consumption (kWh); \( V \) is the voltage (V); \( I \) is the current intensity (A), \( t \) is the time (h) and \( v \) is the volume of the culture (m\(^3\)).

At a fixed value of current, the voltage needed will vary with the resistance offered by the culture medium; if the resistance offered by the culture medium is low, the voltage required will also be low, reducing the energy costs. The optimum values of current intensity, voltage and time can vary depending on the surface charge of the microalgae (zeta potential) that needs to be neutralized.

The electroflocculation time needs to be reduced in order to the overall consumed resources may be lower.

**Electrode material**

In large scale harvesting of *Dunaliella salina*, the electrode material plays an important role in the removal efficiency, cost, downstream treatment and reuse of the culture medium. Therefore, it is important to have in consideration that the electrode material should not interfere with the safety of the microalgae biomass collected and with their growth when the supernatant is reused. When a sacrificial electrode is used, it occurs the anode depletion, whose main disadvantages are the periodical need for replacement and/or cleaning and the metallic contamination of the harvested microalgae and the culture medium (Misra *et al.* 2014; Wiemers & Kohlheb 2014). The use of non sacrificial electrodes avoids metallic contamination, need for replacement and operating time associated with conventional sacrificial electrodes reducing also the energy costs.

Graphite has been suggested as a good option for non sacrificial electrode material due to its excellent electrical conductivity, good thermal shock resistance, high mechanical strength, and low coefficients of thermal expansion and friction (Wiemers & Kohlheb 2014). Besides, graphite has lower price when compared with other non sacrificial electrode materials as platinum and titanium.
Comparing the price of graphite and sacrificial electrodes, as iron and aluminum, there is a large difference in price. Whereas the polished graphite electrodes have a cost of 60 € (2 units with 6 cm×4 cm×0,5 cm, Shunck), the aluminum electrodes can cost 2 € (2 units with 6 cm×4 cm×0,5 cm, Wetplatewagon) and the iron electrodes can cost, approximately, 4 € (2 units with 12,5 cm×2 cm×0,12 cm, Miniscience) (Miniscience n.d.; Shunck Portugal n.d.; Wetplatewagon n.d.). However, non sacrificial electrodes have the advantage of not needing frequent replacement.

**Electrode distance**

The distance between electrodes is directly proportional to the voltage required. Therefore, if the distance between electrodes is small, the necessary voltage to maintain the required amount of current intensity is low. However, a smaller volume of culture will be flocculated between these electrodes. The distance between electrodes used in industry is between 0.1-3.0 cm (Malakootian & Yousefi 2009; Phalakornkule et al. 2009). Since the distance between electrodes needs to be short, several electrodes in parallel, for maximum culture flocculation at minimum power requirement, are used in large scale operation (Mameri et al. 1998).

However, at distances lower than 1cm the fluid transference was obstructed, since the accumulated flocs and bubbles between the electrodes lead to an increase in electrical resistance, thereby increasing energy consumption (Phalakornkule et al. 2009). Thus, electrode distances below 1 cm have not been considered in this study.

**Electrode surface area**

The surface area of the electrodes will determine the size of the electroflocculation setup. The higher the electrode surface area, the higher will be the volume between the electrodes, if the distance is kept constant, and a larger volume of *Dunaliella salina* culture will be flocculated. The surface area (s) to volume (v) ratio (s/v) is an important scale-up parameter, and its optimal values are in the range of 15-45 (m²/m³). Therefore, for a large scale electroflocculation setup the electrode surface can be determined by estimating the optimal value of the s/v ratio and the recommended maximum volume of microalgae culture (Mameri et al. 2001).
Stirring speed

The flocculation process depends on collisions among particles in suspension, caused by their relative motion, which may due to Brownian movement (random motion of particles suspended in a fluid), or induced by an external force, e.g. stirring. At lower stirring speeds, a layer of microalgae flocs can float at the surface. However, higher stirring speeds can result in flocs disaggregation as they are not strong enough to withstand high shear forces (Zhanga et al. 2014).

pH

The culture pH is an important factor that influences the efficiency and performance of the electroflocculation process since the microalgae are sensitive to pH changes. Flocculation is favored by higher salinity of microalgae culture and increasing pH, which can occur naturally due to inorganic carbon (CO$_2$) consumption by the photosynthetic activity of the microalgae or by chemical addition (Besson & Guiraud 2013; Sathe 2010).

Several authors state that the optimal pH for electroflocculation ranges from 6 to 8 (Holt et al. 1999; Xu & Zhu 2004). However, Gao et al. 2010 and Vandamme et al. 2011 concluded in their studies that the optimal pH for electroflocculation is below 7.

In this study the pH was not adjusted by chemicals addition. The changes of pH were a consequence of the electrolytic process, i.e., NaOH production.

Some studies reveal that the amount of microalgae flocs formed during the electroflocculation, using non sacrificial electrodes, increases with the amount of NaOH in solution, which suggests that the flocs’ formation was caused by the precipitation of compounds from the culture medium. The pH increase induces the formation/precipitation of magnesium hydroxide, which according to the results of recent studies, improve the harvesting efficiency (Vandamme et al. 2012). Folkman & Wachs 1973 showed that the positively charged magnesium hydroxide was the main cause for charge neutralization in the electroflocculation process. This flocculation mechanism induced by pH can be considered as an indirect mechanism, since the NaOH formed is not the flocculant itself (Vandamme et al. 2013).

The recycling of the culture medium is not compromised by the presence of NaOH because moderately high pH values can be beneficial for the yield of β-carotene production (Ben-Amotz & Mori 2012).
Salinity

The salinity is related to the conductivity of the current flow. Thereby, at higher salinities, as in the case of *Dunaliella salina*, less power is consumed to maintain the required amount of current because of the solution high ionic strength.

2.4.1 Cell viability after electroflocculation

The quantification of viable cells may be made by using Fluorescein diacetate (FDA) that is a non-polar ester fluorochrome, which is a fluorescent chemical compound that can re-emit light upon light excitation. Once inside the cell, FDA is hydrolyzed by esterase (an enzyme present in viable cells) to produce fluorescein whose conversion rate is correlated with photosynthesis and nutrient-limited growth. Fluorescein accumulates inside the wall of the viable cells which appear green under UV light, allowing the counting of viable cells (Regel *et al.* 2002).

However, the quantification of cell concentration in the culture medium can be done by using other fluorochromes, to which live cells are not permeable, such as Propidium Iodide (PI), or with specific equipment, such as a Muse® Cell Analyzer or Cellometer®.

PI is a membrane-impermeable dye, which is generally excluded from viable cells, that fluoresces red when bound to DNA by intercalating between the base pairs. PI has excitation/emission maxima of 535/ 617 nm respectively (Thermo Fisher Scientific n.d.).

In this study FDA will be used to quantify the viable cells after electroflocculation.

2.4.2 Harvesting system

There are several systems for harvesting microalgae based on the various separation methods, such as SiC Ceramic membranes, based on cross flow filtration, from LiqTech; Algae Venture Harvesting Systems, based on cross flow filtration with a belt filter, from Algae Ventures; and Vacuum airlift, based on dissolved air flotation, from Coldep (Coldep n.d.; Chohan & Bradley n.d.; LiqTech n.d.).

In this section we will be presented a novel equipment for the microalgae harvesting using electroflocculation, which seems to be promising for large-scale harvesting.

OriginClear Inc., which before 2015 was called OriginOil, provides water treatment and microalgae harvesting solutions. Based on two years of tests at James Cook University in Australia, the Model 4 Algae Appliance™ was conceived, which is able to concentrate 4 L per minute (approximately 6 000 L per day) of algae culture. This apparatus can operate
continuously or intermittently, with a wide variety of microalgae strains, salinities, temperatures and growth mode. This concentration process is continuous, chemical free and does not require too much energy (approximately 0.2 kWh/m$^3$) (OriginOil 2012b) (OriginOil n.d.). Figure 4 shows a schematic representation of the Algae Appliance™ Model 4.

![Figure 4. The Algae Appliance™ Model 4 (OriginOil 2012a).](image)

This apparatus provides a control of the microalgae flocs formation since the electroflocculation parameters can be adjusted in order to obtain microalgae cells adequate for the downstream application required, namely: intact, stressed or completely rupture. To cause the flocculation and cell wall rupture it is used an electromagnetic pulse.

The procedure of microalgae concentration, which is based on the application of electrical fields, is divided in two stages: in the first stage there is the microalgae aggregation and in the second stage there is the flotation and extraction of aggregated cells, as is possible to see in Figure 5.

The present model has 152 cm of length, 66 cm of width and 152 cm of height, it can process 6 000 L per day and weights 181 kg (OriginOil n.d.). It has a suggested a price of, approximately, 45 000 € (OriginOil 2012b). The company have a patent of the process entitled “Harvesting and Dewatering Algae Using a Two-Stage Process US 20130228464 A1” (Eckelberry & Sanchez 2013).
This equipment appear to be promising since also exist other models, e.g. Algae Appliance model 200, that can deal with flow rates up to 200 L/min, approximately 290 000 L per day, and harvesting efficiency of the microalgae biomass up to 95%. Therefore, taking into account the normal salt pans volume to be treated is possible to choose which model is more suitable.
Evaluation of electroflocculation for harvesting Dunaliella salina using non sacrificial electrodes
Chapter 3: Materials and Methods

3.1 Materials and equipment

For the assays carried out during this study the following materials and equipment were used: 2 graphite electrodes (6 cm x 4 cm x 0.5 cm, HK1, 97% m/m purity, Shunck), glass beaker (600 mL), mercury thermometer (Nahita), magnetic stirrer bar (2.5 cm), magnetic stirrer (Model F 30, Falc), DC power supply (model AX 503, maximum 30 V and 2.5 A, Metrix), spectrophotometer UV (V-1200, VWR), pH meter (pH 212, Hannacom instruments), microprocessor conductivity meter (RE 387 Tx, EDT Instruments), stainless steel wire (Ni Cr 8020, Cronix extra, 1 mm of diameter, VDM), electronic precision balance (precision of 0.1 mg, Acculab), Leica microscope (Model DM LB2, camera DFC 300, LAS V4.2 software, Leica Instruments), graded wooden bar, Neubauer Haemacytometer (specifications: 0.1 mm of depth, 0.0025 mm$^2$ of a small square area; Optik Labor), centrifuge (model 5810 R, Eppendorf) and vortex (VWR V3, USA).

3.2 Solutions, solvents and reagents

During analysis and assays the following solvents were used: nitric acid ($\geq$65%, Sigma Aldrich) acetone ($\geq$99.5%, Sigma Aldrich), distilled water and FDA reagent in powder form (Sigma-Aldrich).

In the following sections the generic procedures to make the solutions used in this work are presented.

3.2.1 Nitric acid 0.5 M solution

The 0.5 M solution of nitric acid was obtained by mixing 6.4 mL of nitric acid with 193.6 mL of distilled water. This solution can be stored at room temperature during a period of 12 months.
3.2.2 2% FDA solution

The 2% FDA solution was obtained by weighting 20 mg of FDA powder dissolving it with 1 mL of acetone in order to obtain a solution of 2% m/v of FDA, which was then homogenized. This solution can be stored, at -20°C in the dark during 3 months.

3.3 Experimental procedure

3.3.1 Dunaliella salina culture

The *Dunaliella salina* laboratory cultures used in the experimental work were cultivated, by A4F, in 1-6 L flasks in hypersaline artificial salt water adjusted to 120 g/L of NaCl. Cultures growth is in a temperature controlled chamber at 24 °C, under continuous light submitted to an irradiance between 150-300 µmol.m⁻².s⁻¹. Cultures were submitted to constant air flow with 2% CO₂ in order to maintain the pH between 7 and 8. Outdoor cultures, used in the preliminary tests, were cultivated in 60 L flat panel photobioreactors (Green-Wall, GW) and the same hypersaline medium was used. However, these cultures were submitted to natural light and photoperiod. The pH of all cultures was kept between 7 and 8 through addition of CO₂. The culture temperature was kept below 20°C. The orange cultures used in this study were cultivated under these conditions in a depleted nitrogen medium.

3.3.2 Electrofloculation

All the electrofloculation experiments were carried out at room temperature in a 600 mL cylindrical glass reactor with 500 mL of *Dunaliella salina* culture. The graphite electrodes were kept in the middle of the reactor and the distance between them was maintained with the help of a graded wood bar. The anode and cathode were connected to the positive and negative poles, respectively, of a DC power supply that allows the regulation of the intensity of the current applied. Figure 6 shows the experimental electrofloculation setup used.
Materials and Methods

The influence of the different parameters analyzed in this study will be compared based on the harvesting efficiency (HE) and power consumption (P). The *Dunaliella salina* harvesting efficiency was calculated based on the decrease in optical density (OD) of the culture. Samples were collected 3 cm below the surface to avoid the layer of *Dunaliella salina* flocs. The OD was measured at 680 nm (Zenouzi *et al.* 2013).

The harvesting efficiency can be calculated using equation 11 (Vandamme *et al.* 2011):

\[
HE (\%) = \frac{OD_b (680) - OD_a (680)}{OD_b (680)} \times 100
\]  

(11)

where, $OD_b$ is the OD of the sample prior to the electroflocculation and $OD_a$ is the OD of the sample at a defined electroflocculation time.

The electroflocculation power requirement (P) can be calculated using equation 10.

The flocs and impurities that accumulate in the surface of the cathode after electroflocculation were removed by cleaning the cathode with deionized water. However, after every 3 assays the cathode was cleaned with nitric acid solution of 0.5 M for a more efficient removal of the precipitate accumulated in the cathode.

3.3.2.1 Preliminary tests

Prior to the study of the influence of different parameters on the electroflocculation process, some preliminary tests were carried out to identify the working range of the variables.
that will be studied. The range of the variables to be studied was based on similar studies about microalgae electroflocculation, as is possible to see in Table 4.

<table>
<thead>
<tr>
<th>Algae specie</th>
<th>I (A)</th>
<th>Electrode distance (cm)</th>
<th>Stirring speed (rpm)</th>
<th>EF time (min)</th>
<th>EC (S/m)</th>
<th>Sample volume (L)</th>
<th>Anode material and dimensions</th>
<th>Stirring time after EF (min)</th>
<th>HE (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Dunaliella salina</em></td>
<td>0.9</td>
<td>3</td>
<td>100</td>
<td>7</td>
<td>6.01</td>
<td>0.3</td>
<td>Aluminium, 6 cm²</td>
<td>8</td>
<td>94%</td>
</tr>
<tr>
<td><em>Dunaliella salina</em></td>
<td>0.3</td>
<td>1.29</td>
<td>228</td>
<td>20</td>
<td>-</td>
<td>0.25</td>
<td>Aluminium, 25 cm²</td>
<td>-</td>
<td>94%</td>
</tr>
<tr>
<td><em>Chlorella vulgaris</em></td>
<td>13</td>
<td>2</td>
<td>50</td>
<td>20</td>
<td>-</td>
<td>0.65</td>
<td>Aluminium, 30 cm²</td>
<td>20</td>
<td>&gt;95%</td>
</tr>
<tr>
<td><em>C. sorokiniana and S. obliqua</em></td>
<td>1</td>
<td>6</td>
<td>No stirring</td>
<td>60</td>
<td>-</td>
<td>0.9</td>
<td>Graphite, 120 cm²</td>
<td>-</td>
<td>94.5</td>
</tr>
</tbody>
</table>

Table 4. Experimental parameters of existing microalgae electroflocculation methods.

1 (Zenouzi et al. 2013).
2 (Mohammad-Ghasemnejadmaleki et al. 2014).
3 (Zhang 2014).
4 (Misra 2014).

The experimental program started by carrying out some assays without stirring and varying the following parameters: electrode distance, current intensity and floating time. These preliminary tests showed that there was no formation of visible flakes.

Then the influence of the stirring speed on the harvesting efficiency was studied. The introduction of stirring showed a subtle formation of flocs. However, the formed flocs set down instead of floating. When the stirring was maintained, after the electrical energy was switched off, it was observed the formation/aggregation of homogeneous flocs that, after the stirring was switched off, float up to the surface, leading to a significant decrease of the ODₐ of the culture medium and of the floating time. Thus, the experimental procedure will be divided into 3 steps in order to achieve higher harvesting efficiency. Figure 7 shows the lab-scale experimental setup and the steps to be followed during the electroflocculation procedure. During the first step, agitation and electrical current were on. After a certain period of time, the current was switched off but agitation was kept on in order to achieve a homogeneous flocs formation. The preliminary tests reveal that the optimal time for this second step was 3 minutes. In the last step, both current and agitation were off. The preliminary test reveal that the optimal time for this last step, where the flocs float to the surface, was also 3 minutes.
The influence of the different variables on the electroflocculation was studied using a one-variable-at-a-time approach. Successively, the influence of the electrode distance, electroflocculation time, stirring speed, current intensity and electrical conductivity was investigated. The influence of a specific variable on the electroflocculation process was studied using the best values founded for the previously studied variables. The range of electrode distance that will be studied is between 1 and 5 cm; the range of the electroflocculation time is between 1 and 10 minutes; the range of the stirring speed is between 0 and 600 rpm; the range of the current intensity is between 0.3 and 0.9 A and the electrical conductivity is studied using a microalgae culture with 102, 120 and 146 g/L of NaCl. The results of the study of the influence of these variables are present in Chapter 4, with the same scheme of presentation that the experimental procedure, i.e., by topics.

### 3.3.2.2 Influence of the electrode distance on electroflocculation

i) Measure the $T_b$, $EC_b$, $pH_b$ and $OD_b$ of the initial culture.

ii) Experimental conditions:
   - Electroflocculation time: **6 minutes**
   - Stirring time after electroflocculation: **3 minutes**
   - Floating time: **3 minutes**
   - Current intensity: **0.5 A**
   - Stirring speed: **100 rpm**

iii) Change the distance between the electrodes: 1, 2, 3, 4 and 5 cm; and for each experiment measure the voltage, $T_a$, $EC_a$, $pH_a$ and $OD_a$. 

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**Figure 7.** Designed electroflocculation lab-scale setup from preliminary tests.
Evaluation of electroflocculation for harvesting *Dunaliella salina* using non sacrificial electrodes

3.3.2.3 Influence of the time on electroflocculation

i) Measure the $T_b$, $EC_b$, $pH_b$ and $OD_b$ of the initial culture.

ii) Experimental conditions:
   - Stirring time after electroflocculation: **3 minutes**
   - Floating time: **3 minutes**
   - Current intensity: **0.5 A**
   - Stirring speed: **100 rpm**

iii) Choose a value of electrode distance (optimum value found).

iv) Change the time of electroflocculation to 1, 2, 4, 6, 8 and 10 minutes; and for each experiment measure the voltage, $T_a$, $EC_a$, $pH_a$ and $OD_a$.

3.3.2.4 Influence of the stirring speed on electroflocculation

i) Measure the $T_b$, $EC_b$, $pH_b$ and $OD_b$ of the initial culture.

ii) Experimental conditions:
   - Stirring time after electroflocculation: **3 minutes**
   - Floating time: **3 minutes**
   - Current intensity: **0.5 A**

iii) Choose a value of electrode distance (optimum value found) and time (optimum value found).

iv) Change the stirring speed from 0 to 600 rpm; and for each experiment measure the voltage, $T_a$, $EC_a$, $pH_a$ and $OD_a$.

3.3.2.5 Influence of the current intensity on electroflocculation

i) Measure the $T_b$, $EC_b$, $pH_b$ and $OD_b$ of the initial culture.

ii) Experimental conditions:
   - Stirring time after electroflocculation: **3 minutes**
   - Floating time: **3 minutes**

iii) Choose a value of electrode gap (optimum value found), time (optimum value found) and stirring speed (optimum value found).

iv) Change the intensity of the current applied: 0.3, 0.5, 0.7 and 0.9 A; and for each experiment measure the voltage, $T_a$, $EC_a$, $pH_a$ and $OD_a$. 

Materials and Methods
### 3.3.2.6 Influence of the electrical conductivity on electroflocculation

i) Measure the $T_b$, $EC_b$, $pH_b$ and $OD_b$ of the initial culture.

ii) Experimental conditions:
   - Stirring time after electroflocculation: **3 minutes**
   - Floating time: **3 minutes**

iii) Choose a value of electrode gap (optimum value found), time (optimum value found) and stirring speed (optimum value found).

iv) Change the intensity of the current applied: 0.3, 0.5, 0.7 and 0.9 A.

v) Repeat this procedure with cultures of 102 g/L and 146 g/L of NaCl; and for each experiment measure the voltage, $T_a$, $EC_a$, $pH_a$ and $OD_a$.

### 3.3.2.7 Measurement of cell viability

The measurement of cell viability was made with the previously optimized parameters by analyzing the two distinct liquid phases formed after the electroflocculation: the top layer, formed by the floating flocs, and the culture medium, that remains below the top layer.

After the electroflocculation, the culture medium was removed with the aid of a silicone tube until no more medium could be removed without dragging the flocs in the upper layer, and the removed volume was measured. Then, a known volume of medium with 120 g/L of NaCl (without microalgae) was used to clean the glass beaker and the tube.

Then, 200 µL samples were taken from the top layer and the bottom layer, to which 4 µL of FDA solution was added. After letting react during 5 min, at room temperature, the samples were analyzed using a Leica microscope equipped with the barrier filter BP 515/560 using wavelengths of excitation and emission light of 450 - 490 and 515 - 565 nm, respectively. Cell counting was performed 3 times or until an error lower than 10% between counts was achieved, by using a Neubauer Haemacytometer. The viable cell concentration was calculated based on both top and bottom viable cells after electroflocculation (Figure 2-b). The total viable cell concentration after electroflocculation was calculated based on equation 12:

$$C_{Total} = \frac{C_{Top}V_{Top} + C_{Bottom}V_{Bottom}}{V_{Top} + V_{Bottom}}$$  \hspace{1cm} (12)

This test was made after the study of both effect of electrical current and stirring speed on electroflocculation.
Materials and Methods
Chapter 4: Results and Discussion

In this chapter is described the influence of several variables in the electroflocculation process, including: electrode distance, electroflocculation time, stirring speed, current intensity and electrical conductivity. This influence was compared in terms of harvesting efficiency and power consumption.

The experimental results are presented by topics in the following sections.

4.1 Preliminary tests

The preliminary tests were carried out using a brown phase *Dunaliella salina* culture – an intermediate phase between a green and orange phase – cultivated outside, in A4F pilot unit. The brown phase culture used had a carotenoids/chlorophylls (car/chl) ratio of 2.5, which is an intermediate value between a green phase culture – 0.2 due to the high value of chlorophylls - and an orange phase culture – 10 due to the high value of carotenoids. The culture used had a cell concentration of \(7.6 \times 10^5\) cells/mL, and a OD\(_b\) of 0.380. A figure showing the initial brown culture of *Dunaliella salina* is given in Appendix 2.1 (Figure 15-a). Initially, the electroflocculation process was tested without stirring, keeping the electrodes 3 cm apart, varying the electroflocculation time between 3 and 6 minutes, the floating time between 0 and 20 minutes and the current intensity between 0.3 and 0.9 A. Using these parameters, there was no formation of visible flocs and the OD\(_a\) remained practically the same after the electroflocculation, as is possible to see in Figure 15-b in (Appendix 2.1). After 20 minutes of floating time, there were no significant changes in OD\(_a\), having only occurred a slight microalgae sedimentation, which occurs naturally.

In a second phase, the electroflocculation was tested with stirring, approximately 100 rpm, keeping the electrode distance constant, approximately 3 cm, the electroflocculation time between 3 and 6 minutes, the floating time between 0 and 20 minutes and changing the current intensity between 0.3 and 0.9 A. Using these parameters, it was observed a subtle formation of flocs. However, the formed flocs do not ascend to the surface. When the stirring was maintained during 3 minutes, after the electrical current was switched off, the formation/aggregation of homogeneous flocs is promoted, and after the stirring was switched off, they float up to the surface after 3 minutes, decreasing considerably the OD\(_a\) of the culture medium, as is possible to see in Figure 15-c (Appendix 2.1). Thus, this led to divide the experimental procedure into 3 steps in order to achieve higher harvesting efficiency, as shown in Figure 7.
In what follows, it will be used an orange phase *Dunaliella salina* culture cultivated in the A4F laboratory as described previously, in order to reduce the impact of the presence of typical outdoor cultivation contaminants such as fungi and bacteria and ensure that the concentration of each batch remains, approximately, the same. In Figure 16 (Appendix 2.2) is shown a microscope image of the initial orange culture that was used in this study.

### 4.2 Influence of the electrode distance on electroflocculation

The effect of the distance between electrodes on optical density (a), energy consumption (b) and harvesting efficiency (c) of *Dunaliella salina* culture with 120 g/L of NaCl, at fixed current intensity of 0.5 A, electroflocculation time of 6 min, stirring speed of 100 rpm, stirring time after electroflocculation of 3 min and floating time of 3 min is shown in Figure 8. The concentration of the *Dunaliella salina* culture used was 2.3×10^5 cells/mL, the car/chl ratio was 5.9 and the OD₅₅₀ was 0.195. The lowest OD₅₅₀ for the *Dunaliella salina* suspension was achieved when the electrodes were at 1 cm from each other (Figure 8-a). The present results suggest that the further away the electrodes are placed the highest is the optical density measured, which tends to stabilize (Table 5). This means that when the electrodes were adjusted closer to each other, the electroflocculation process occurred more efficiently, achieving a harvesting efficiency of 39.8% (Figure 8-c).

Figure 8-b shows that when the electrode distance increases, the energy consumption also increases from 0.38 kWh/m³ to 0.46 kWh/m³, which corresponds to an increase of 15.8%. This occurs because when the electrodes distance increases the required voltage to maintain the required amount of current flow also increases, due to the increase of the medium resistance (Voltage = Resistance × Current intensity). According to equation 10, when the voltage increases, and the current and time remain constant, the power consumption increases.

The T, pH and EC of the *Dunaliella salina* culture were measured at the beginning and at the end of each assay. The initial culture temperature was, approximately, 18 °C, increasing about 1.5 °C during each experiment. The maximum increase in temperature was of 11.7%. Although the stirring speed used was low it can be the cause for this slight temperature increase. To confirm that, a test was carried out by stirring water at 100 rpm (stirring velocity used in the study of the influence of the distance between the electrodes) during the same period of time but without the electrodes. It was found an increase of 1.5°C, which confirms that the temperature increase is mainly due to the stirring process.
The pH of the initial culture was 6.58 and increased until 7.68. The increase was progressive from the smallest to the largest distance between the electrodes. This effect is due to the fact that when the electrodes distance increases the necessary voltage to maintain the required amount of current flow also increases, which results in a higher dissociation of the water molecules and consequent formation of stronger bases, as NaOH (Chaplin 2016).

The EC of the initial culture was 9.94 S/m and increased until 10.20 S/m, being this increase also progressive from the smallest to the largest distance between the electrodes and corresponds to an increase of 2.62%. This increase is related to the pH increase because the molar conductivity of NaOH is greater than the molar conductivity of NaCl, thereby causing an increment of electrical conductivity (Artemov et al. 2015).

For the following tests, it was chosen the value of electrode distance that achieved both high harvesting efficiency and low power consumption, i.e., 1 cm.

**Figure 8.** Results of the effect of the electrode distance on (a) optical density, (b) power consumption and (c) harvesting efficiency of *Dunaliella salina* culture with 120g/L.
Table 5. Results of HE (%) and P (kWh/m$^3$) obtained from the study about the influence of the electrode distance on electroflocculation.

<table>
<thead>
<tr>
<th>I (A)</th>
<th>V (Volt)</th>
<th>Electrode distance (cm)</th>
<th>OD$_b$</th>
<th>OD$_a$</th>
<th>HE (%)</th>
<th>P (kWh/m$^3$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.8</td>
<td>1</td>
<td>0.117</td>
<td>39.8</td>
<td>0.38</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.9</td>
<td>2</td>
<td>0.134</td>
<td>31.1</td>
<td>0.39</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.5</td>
<td>4.0</td>
<td>3.0195</td>
<td>0.152</td>
<td>22.1</td>
<td>0.40</td>
<td></td>
</tr>
<tr>
<td>4.3</td>
<td>4</td>
<td>0.153</td>
<td>21.4</td>
<td>0.43</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4.6</td>
<td>5</td>
<td>0.149</td>
<td>23.4</td>
<td>0.46</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

4.3 Influence of time on electroflocculation

The effect of the electroflocculation time on optical density (a), energy consumption (b) and harvesting efficiency (c) of *Dunaliella salina* culture with 120g/L of NaCl, at fixed electrode distance of 1 cm, current intensity of 0.5 A, stirring speed of 100 rpm, stirring time after electroflocculation of 3 min and floating time of 3 min is shown in Figure 9. The concentration of the *Dunaliella salina* culture used was $2.3 \times 10^5$ cells/mL, the car/chl ratio was 5.9 and the OD$_b$ was 0.195. The lowest OD$_a$ for the *Dunaliella salina* suspension after electroflocculation was achieved when the electroflocculation was carried out during 6 minutes (Figure 9-a). After that, and using the fixed parameters described above, there was a decrease of the harvesting efficiency, which may be caused by the formation of Mg(OH)$_2$ during the experiment, that became metastable and the flocs flotation becomes more difficult, thus the *Dunaliella salina* flocs remain in solution and the OD$_a$ increase (Table 6) (Xiong *et al.* 2015). The higher electroflocculation harvesting efficiency achieved was 33.0%, corresponding to an electroflocculation time of 6 minutes (Figure 9-c).

Figure 9-b shows that when the electroflocculation time increases, the energy consumption also increases from 0.06 kWh/m$^3$ to 0.67 kWh/m$^3$, which corresponds to an increase of 1 067%. According to equation 10, when the electroflocculation time increases and the current and voltage remains constant, the power consumption also increases.

In the assays it was also monitored the T, pH and EC of the *Dunaliella salina* culture before and after each experiment. The initial temperature of the culture was, approximately, 18ºC and increased, progressively, by approximately 2ºC, which correspond to a maximum increase of 11.7%. The use of stirring can cause a slight increase in temperature and when applied during a long period of time, this increase may be more noticeable.
The pH of the initial culture was 6.34 and increased, progressively, until 7.78 from the lower to the highest electroflocculation time. When the electroflocculation time increases the H₂O dissociation also increases, promoting a higher formation of NaOH (Chaplin 2016).

The EC of the initial culture was 9.98 S/m and increased progressively until 10.30 S/m from the lowest to the highest electroflocculation time, leading to an increase of 3.21%. This increase is related, as referred above, to the increase of NaOH in solution (Artemov et al. 2015).

Based on the results of harvesting efficiency and power consumption, the best result was achieved by applying electricity during 6 minutes. This was the value of the electroflocculation time used in the following experiments.

**Figure 9.** Results of the effect of the electroflocculation time on (a) optical density; (b) power consumption and (c) harvesting efficiency of *Dunaliella salina* culture with 120g/L of NaCl.
Results and Discussion

Table 6. Results of HE (%) and P (kWh/m$^3$) obtained from the study about the influence of the time on electroflocculation.

<table>
<thead>
<tr>
<th>I (A)</th>
<th>V (Volt)</th>
<th>EF time (min)</th>
<th>OD$_b$</th>
<th>OD$_a$</th>
<th>HE (%)</th>
<th>P (kWh/m$^3$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5</td>
<td>3.8</td>
<td>1</td>
<td>0.204</td>
<td>0.0</td>
<td>0.06</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4.0</td>
<td>2</td>
<td>0.194</td>
<td>0.5</td>
<td>0.13</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4.0</td>
<td>4</td>
<td>0.179</td>
<td>8.4</td>
<td>0.27</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3.9</td>
<td>6</td>
<td>0.131</td>
<td>33.0</td>
<td>0.39</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3.8</td>
<td>8</td>
<td>0.146</td>
<td>25.0</td>
<td>0.51</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4.0</td>
<td>10</td>
<td>0.149</td>
<td>23.8</td>
<td>0.67</td>
<td></td>
</tr>
</tbody>
</table>

4.4 Influence of the stirring speed on electroflocculation

The effects of the stirring speed on optical density (a), energy consumption (b) and harvesting efficiency (c) of Dunaliella salina culture with 120g/L of NaCl, at fixed electrode distance of 1 cm, current intensity of 0.5 A, electroflocculation time of 6 min, stirring time after electroflocculation of 3 min and floating time of 3 min, is shown in Figure 10. The concentration of the Dunaliella salina culture used was 4.2×10$^5$ cells/mL, the car/chl ratio was 5.0 and the OD$_b$ was 0.286. The lowest OD$_a$ of Dunaliella salina suspension was achieved for a stirring speed of 400 rpm (Figure 10-a). From Figure 10-a is possible to see that the increase of stirring speed leads to a decrease in optical density. However, for stirring speeds above 400 rpm the optical density increased (Table 7). This may be due to the fact that below 400 rpm flocs were homogeneously formed and were not disaggregated. However, these flocs may not be strong enough to withstand high shear forces such as stirring velocities higher than 400 rpm (Zhanga et al. 2014). Thus, the electroflocculation process occurred more efficiently at 400 rpm achieving a harvesting efficiency of 69.6% (Figure 10-c).

Figure 10-b shows that the energy consumption varies with the stirring speed used. The power consumption increased with the increase of the stirring speed, from 0.24 kWh/m$^3$ to 0.39 kWh/m$^3$, which corresponds to an increase of 62.5%. However, above 400 rpm the power consumption remains practically the same, as the voltage needed to maintain the current flow was not changed, that may be due to the good medium homogenization.

The T, pH and EC were measured before and after each experiment. The initial temperature of the culture was, approximately, 23°C and increased progressively from the smallest to the highest stirring speed applied, reaching 25°C, the maximum observed increase in temperature was 6.9%. As already discussed, the use of stirring speed can cause a slight increase in temperature and when a higher speed is applied, this increase will be more
noticeable. To confirm that, some tests were carried out using water and different stirring speeds: 100, 200, 300, 400, 500 and 600 rpm during the same period of time but without using the electrodes, which showed a progressive increase of temperature, as verified in the study of the influence of the stirring speed on harvesting efficiency, of 2°C.

The pH of the initial culture was 7.33 and increased until 8.36. The increase was progressive from the smallest to the highest stirring speed due to the higher homogenization of the solution, which leads to a more efficient transport of the electrolytes in solutions, and consequent formation of NaOH (Chaplin 2016).

The EC of the initial culture was 10.10 S/m and increased until 10.65 S/m, which corresponds to an increase of 8.4%. This increase was progressive from the smallest to the highest stirring speed applied, and is related to the pH increase (Artemov et al. 2015).

For following tests, it will be used the stirring speed that allows to achieve both the highest harvesting efficiency and the lowest power consumption, i.e., 400 rpm.

![Graphs](image)

**Figure 10.** Results of the effect of the stirring speed on (a) optical density; (b) power consumption and (c) harvesting efficiency of *Dunaliella salina* culture with 120g/L of NaCl.
Table 7. Results of HE (%) and P (kWh/m³) obtained from the study about the influence of the stirring speed on electroflocculation.

<table>
<thead>
<tr>
<th>I (A)</th>
<th>V (Volt)</th>
<th>Stirring speed (rpm)</th>
<th>OD_b</th>
<th>OD_a</th>
<th>HE (%)</th>
<th>P (kWh/m³)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.4</td>
<td>0</td>
<td>0.232</td>
<td>19.0</td>
<td>0.24</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.3</td>
<td>100</td>
<td>0.207</td>
<td>27.7</td>
<td>0.33</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.4</td>
<td>200</td>
<td>0.195</td>
<td>31.8</td>
<td>0.34</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.7</td>
<td>300</td>
<td>0.170</td>
<td>40.5</td>
<td>0.37</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.9</td>
<td>400</td>
<td>0.087</td>
<td>69.6</td>
<td>0.39</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.9</td>
<td>500</td>
<td>0.226</td>
<td>21.0</td>
<td>0.39</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.8</td>
<td>600</td>
<td>0.228</td>
<td>20.5</td>
<td>0.38</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

4.5 Influence of the current intensity on electroflocculation

The effect of the current flow on optical density (a), energy consumption (b) and harvesting efficiency (c) of *Dunaliella salina* suspension with 120g/L of NaCl, at fixed electrode distance of 1 cm, electroflocculation time of 6 min, stirring speed of 400 rpm, stirring time after electroflocculation of 3 min and floating time of 3 min, is shown in Figure 11. The concentration of the *Dunaliella salina* culture used was $3.6\times10^5$ cells/mL, the car/chl ratio was 5.9 and the OD_b was 0.290. The current range begins at 0.3 A, because at lower values the electroflocculation rate would be low and the electroflocculation time would be too long, therefore the electroflocculation process would be very inefficient (Sathe 2010).

The lowest OD_a of *Dunaliella salina* suspension was achieved for current intensity of 0.7 A (Figure 11-a). It appears that the increase of current intensity leads to a decrease in OD_a since higher current intensity leads to a higher transfer of ions through the solution (Zhanga et al. 2014). However, to higher current intensities of 0.7 A the OD_a increased (Table 8). Thus, the electroflocculation process occurred more efficiently when was used 0.7 A, achieving a harvesting efficiency of 71.1% (Figure 11-c).

When a current intensity of 0.9 A was used, it was observed that the OD_a increased and the color of the final solution was changed from orange to brown-black, as is possible to see in Figure 22 (Appendix 2.5). The reason why this happened is because there is the formation of iron (II) hydroxide (Fe(OH)_2) (green coloration) in an alkaline medium with NaOH that can easily be reduced and form iron (III) hydroxide (Fe(OH)_3) (brown-black color). The iron ion is naturally in the culture medium since its addition is an essential micronutrient for *Dunaliella salina* growth. However, the concentration of iron ion is very low for such reaction occur at significant extend in the culture medium. To confirm this, an
electroflocculation test with hypersaline water (120 g/L) was carried out under the same previous conditions, namely a current intensity of 0.9 A. This assay show the formation of a green-yellow product that seemed to be formed in the connection of the anode with the stainless steel wire (that contains iron in its composition) that became brown-black after the procedure, as it is possible to see in Figure 23 (Appendix 2.5). Besides, after the experiments it was observed that the stainless steel wire became thinner eventually breaking, confirming the hypothesis that the iron ions are originated from it.

The occurring reaction is shown in equation 13. The product formed, Fe(OH)$_2$ (s), can be reduced and form Fe(OH)$_3$ (s), giving rise to a dark brown rusty solid flocs, in an alkaline medium, as is shown by equation 14 (Liu & Millero 1999).

\[
Fe^{2+} (aq) + 2OH^- (aq) \rightarrow Fe(OH)_2 (s) \tag{13}
\]

\[
Fe^{3+} (aq) + 3OH^- (aq) \rightarrow Fe(OH)_3 (s) \tag{14}
\]

Figure 11-b shows that the energy consumption increases with the increase of current intensity applied, from 0.14 kWh/m$^3$ to 1.01 kWh/m$^3$, based on the equation 10, which correspond to an increase of 621%.

The T, pH and EC were measured before and after each experiment. The initial temperature of the culture was, approximately, 21ºC and increased about 1ºC during each experiment, which correspond to an increase of 5.3%. This increase reveals that when the stirring speed and time remains constant, the increase of temperature may be explained by the stirring (i.e., due to the friction caused by the magnet bar).

The pH of the initial culture was 6.60 and increased, progressively, until 8.90 as the current intensity varied from 0.3 A up to 0.7 A. This increase in pH is due to the higher dissolution of ions when higher current intensity is applied, which results in an increase of NaOH formation (Chaplin 2016). However, when a current intensity of 0.9 A was applied the pH only increase until 8.10, which corresponds to an increase, comparing with the initial value, of 22.73%, that is due to the consumption of OH$^-$ in the iron (II) and iron (III) hydroxide formation (Liu & Millero 1999).

The EC of the initial culture was 9.91 S/m and increased, progressively, until 10.28 S/m when the applied a current intensity varied from 0.3 A up to 0.7 A, having been observed a maximum increase of 3.73%. This increase is related to the pH increase, as mentioned above (Artemov et al. 2015). However, the EC decrease when a current intensity 0.9 A was applied,
due to the pH decrease, having been observed an increase comparing with the initial value of 3.03%.

This test concludes the optimization of the electrofloculation parameters. Using an electrode distance of 1 cm, an electrofloculation time of 6 min, a stirring speed of 400 rpm and a current intensity of 0.7 A is possible to achieve 71.1% of harvesting efficiency with a power consumption of 0.63 kWh/m³.

Comparing these results with other electrofloculation studies, the harvesting efficiency was lower and power consumption was higher than expected, once a level of 90-95% of microalgae removal was reported for a power consumption as low as 0.331 kWh/m³ for various microalgae species including green phase *Dunaliella salina* culture (Danquah *et al.* 2009; Poelman *et al.* 1996; Zenouzi *et al.* 2013). However, comparing with other dewatering techniques, the power consumption was lower (Table 2).

![Graphs showing the effect of current intensity on optical density, power consumption, and harvesting efficiency](image_url)

**Figure 11.** Results of the effect of the current intensity on (a) optical density, (b) power consumption and (c) harvesting efficiency of *Dunaliella salina* culture with 120g/L of NaCl.
Table 8. Results of HE (%) and P (kWh/m³) obtained from the study about the influence of the current intensity on electroflocculation.

<table>
<thead>
<tr>
<th>I (A)</th>
<th>V (Volt)</th>
<th>OD_b</th>
<th>OD_a</th>
<th>HE (%)</th>
<th>P (kWh/m³)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.3</td>
<td>2.3</td>
<td>0.288</td>
<td>0.7</td>
<td>0.14</td>
<td></td>
</tr>
<tr>
<td>0.5</td>
<td>3.5</td>
<td>0.170</td>
<td>41.5</td>
<td>0.35</td>
<td></td>
</tr>
<tr>
<td>0.7</td>
<td>4.5</td>
<td>0.084</td>
<td>71.1</td>
<td>0.63</td>
<td></td>
</tr>
<tr>
<td>0.9</td>
<td>5.6</td>
<td>0.227</td>
<td>21.7</td>
<td>1.01</td>
<td></td>
</tr>
</tbody>
</table>

4.6 Influence of the electrical conductivity on electroflocculation

The effect of the electrical conductivity on optical density (a), energy consumption (b) and harvesting efficiency (c) of Dunaliella salina orange culture with 102, 120 and 146 g/L of NaCl, at fixed electrode distance of 1 cm, electroflocculation time of 6 min, stirring time after electroflocculation of 3 min, floating time of 3 min and stirring speed of 400 rpm, is shown in Figure 12.

The cell concentration of the Dunaliella salina culture with 102 g/L of NaCl was $4.6 \times 10^5$ cells/mL, the car/chl ratio was 5.6 and the OD_b was 0.243; the concentration of the Dunaliella salina culture with 120 g/L of NaCl was $4.2 \times 10^5$ cells/mL, the car/chl ratio was 5.0 and the OD_b was 0.270; and the concentration of the Dunaliella salina culture with 146 g/L of NaCl was $4.0 \times 10^5$ cells/mL, the car/chl ratio was 5.3 and the OD_b was 0.223. The differences on OD_b between the different cultures are due to the fact that they have different car/chl ratios, having different absorbance peaks, hence the OD can vary even for almost equal cell concentrations. As the OD_b of the three cultures were different, it will only be compared the harvesting efficiency between each other.

From Figure 12-c is possible to see that the harvesting efficiency is different for different NaCl concentrations. The presence of high amounts of NaCl leads to the increase of the solution ionic strength, which makes easier the water molecules dissolution. Therefore, the harvesting efficiency increases with the increase of NaCl concentration (Uduman et al. 2011). Figure 12-c show that the lowest harvesting efficiency was achieved using the culture with lower NaCl concentration (102 g/L of NaCl), which corresponds to a harvesting efficiency of 62.7%, and the highest harvesting efficiency was achieved using the culture with 120 g/L NaCl, which corresponds to a harvesting efficiency of 78.5% (Table 9 and 10). The harvesting efficiency of the culture with 146 g/L of NaCl was 70.4%, which was lower than the culture with 120 g/L of NaCl (Table 11). This can be explained by the fact that when the salinity of the medium increases, the microalgae Dunaliella salina responds by changing its
metabolism, increasing the uptake of CO₂ with the subsequent synthesis of glycerol. Moreover, the induced stress by increasing the salinity causes a change in the protein synthesis and consequent alteration of protein and lipids contents of the plasmatic membrane, this change in the membrane properties may influence the microalgae charge neutralization (Alkayal et al. 2010; Lüttge et al. 1993; Katz et al. 2007).

However, no conclusions can be drawn about the relationship between the harvesting efficiency and the salinity of the medium since were used cultures with different cell concentration and the parameters that were applied were optimized for a culture with 120 g/L of NaCl, which may defer from the optimum values for the other NaCl concentrations.

Figure 12-b shows the energy consumption for the different cultures. It was observed that, for all cultures, the energy consumption increased with increasing current intensity. However, this increase was more noticeable in the solution with lower EC. The voltage that is necessary to maintain the current flow of the culture with 102 g/L of NaCl increased from 3.3 up to 4.9 V, varied from 0.20 kWh/m³ up to 0.88 kWh/m³, which corresponds to an increase of 340%; The necessary voltage to maintain the current flow of the culture with 120 g/L NaCl increased from 3.2 up to 4.3 V, leading to an increase of power consumption of 0.19 kWh/m³ to 0.77 kWh/m³, which corresponds to an increase of 305%. The voltage that is necessary to maintain the current flow of the culture with 146 g/L NaCl increased from 3.1 up to 3.9 V and the power consumption increased from 0.19 kWh/m³ to 0.70 kWh/m³, which corresponds to an increase of 268%. From this study it can be concluded that harvesting microalgae in a solution with higher EC (i.e., saltwater) will consume lower energy that a solution with lower EC (freshwater). This is due to the high ionic strength of saltwater (Sathe 2010; Zenouzi et al. 2013).

At the begin and the end of each experiment it was measured the T, pH and EC of the cultures. The initial temperature of the culture increased 1ºC during the electroflocculation process, in all experiments. This increase can be explained by the stirring of the culture (400 rpm).

The pH of the culture with 102 g/L of NaCl was 7.04 and increased, progressively, until 7.66 with increasing current intensity; the pH of the culture with 120 g/L NaCl was 6.99 and increased, progressively, until 7.91 from the smallest to the highest current intensity applied; and the pH of the culture with 146 g/L NaCl was 6.92 and increase, progressively, until 7.99 with increasing current intensity. This is due to the fact that in stronger ionic solutions the H₂O dissociation is higher, which is essential for NaOH formation (Chaplin 2016).
The initial EC of the culture with 102 g/L of NaCl was 9.05 S/m and the final was 9.82 S/m, which corresponds to an increase of 8.5%; the culture with 120 g/L NaCl had an initial EC of 10.22 S/m and a final EC of 10.41 S/m, which corresponds to an increase of 1.9%; for the culture with 146 g/L NaCl the initial EC was 10.80 S/m and the final EC 11.50 S/m, which corresponds to an increase of 6.4%. The final EC of the solutions described was higher than the initial values. This increase is related to the pH increase, due to the greater molar conductivity of NaOH (Artemov et al. 2015).

![Graphs](image)

**Figure 12.** Results of the effect of the EC on (a) optical density, (b) power consumption and (c) harvesting efficiency of *Dunaliella salina* culture with 102, 120 and 146 g/L of NaCl.

**Table 9.** Results of HE (%) and P (kWh/m³) obtained from the study about the influence of the electrical conductivity on electroflocculation, using a *Dunaliella salina* culture with 102 g/L of NaCl.

<table>
<thead>
<tr>
<th>I (A)</th>
<th>V (Volt)</th>
<th>OD&lt;sub&gt;b&lt;/sub&gt;</th>
<th>OD&lt;sub&gt;a&lt;/sub&gt;</th>
<th>HE (%)</th>
<th>P (kWh/m³)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.3</td>
<td>3.3</td>
<td>0.197</td>
<td></td>
<td>19.2</td>
<td>0.20</td>
</tr>
<tr>
<td>0.5</td>
<td>4.1</td>
<td></td>
<td>0.178</td>
<td>26.7</td>
<td>0.41</td>
</tr>
<tr>
<td>0.7</td>
<td>4.3</td>
<td>0.243</td>
<td></td>
<td>43.0</td>
<td>0.60</td>
</tr>
<tr>
<td>0.9</td>
<td>4.9</td>
<td>0.091</td>
<td></td>
<td>62.7</td>
<td>0.88</td>
</tr>
</tbody>
</table>
Table 10. Results of HE (%) and P (kWh/m$^3$) obtained from the study about the influence of the electrical conductivity on electroflocculation, using a Dunaliella salina culture with 120 g/L of NaCl.

<table>
<thead>
<tr>
<th>I (A)</th>
<th>V (Volt)</th>
<th>OD$_b$</th>
<th>OD$_a$</th>
<th>HE (%)</th>
<th>P (kWh/m$^3$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.3</td>
<td>3.1</td>
<td>0.165</td>
<td>0.172</td>
<td>26.3</td>
<td>0.19</td>
</tr>
<tr>
<td>0.5</td>
<td>3.5</td>
<td>0.157</td>
<td>0.165</td>
<td>29.6</td>
<td>0.35</td>
</tr>
<tr>
<td>0.7</td>
<td>3.7</td>
<td>0.123</td>
<td>0.128</td>
<td>45.1</td>
<td>0.52</td>
</tr>
<tr>
<td>0.9</td>
<td>3.9</td>
<td>0.066</td>
<td>0.072</td>
<td>70.4</td>
<td>0.70</td>
</tr>
</tbody>
</table>

Table 11. Results of HE (%) and P (kWh/m$^3$) obtained from the study about the influence of the electrical conductivity on electroflocculation, using a Dunaliella salina culture with 146 g/L of NaCl.

<table>
<thead>
<tr>
<th>I (A)</th>
<th>V (Volt)</th>
<th>OD$_b$</th>
<th>OD$_a$</th>
<th>HE (%)</th>
<th>P (kWh/m$^3$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.3</td>
<td>3.2</td>
<td>0.148</td>
<td>0.153</td>
<td>45.2</td>
<td>0.19</td>
</tr>
<tr>
<td>0.5</td>
<td>3.7</td>
<td>0.127</td>
<td>0.132</td>
<td>52.9</td>
<td>0.37</td>
</tr>
<tr>
<td>0.7</td>
<td>3.9</td>
<td>0.118</td>
<td>0.119</td>
<td>56.2</td>
<td>0.55</td>
</tr>
<tr>
<td>0.9</td>
<td>4.3</td>
<td>0.058</td>
<td>0.063</td>
<td>78.5</td>
<td>0.77</td>
</tr>
</tbody>
</table>

4.7 Measurement of cell viability

The electroflocculation parameters optimization for the harvesting of Dunaliella salina was done based on the highest harvesting efficiency and lower power consumption. However, it is also important to take into account the cell viability and cell morphology after the assays since it may affect the β-carotene extraction procedure and efficiency, which is the next step. As a complementary measure to the OD, it was studied the cell viability by microscopy using FDA as fluorochrome.

Cell counting was performed by counting the green and green tones cells in the sample, which correspond to viable cells. There were some red cells in the samples that were not counted, as their color is due to Dunaliella salina auto fluorescence. However, and as explained below, is not possible to conclude without any doubt, that these red cells corresponds to unviable cells, since they can simply be “waterproofed” to FDA due to the formation of a mucus layer surrounding the cell. Thus, the influence of these red cells in the balance of total cell concentration will be discussed. Figure 32 (Appendix 2.1) shows a green and red cell in a sample.

In Tables 12 and 13 are shown the calculated cell concentration of the top layer (top) and underneath (bottom) for each assay. The total viable cell concentration (C$_{total}$) was calculated based on equation 12.
Table 12. Result of the cell counting varying the current intensity.

<table>
<thead>
<tr>
<th>I (A)</th>
<th>V (volt)</th>
<th>Stirring speed (rpm)</th>
<th>( C_{\text{top}} ) (cells/mL)</th>
<th>( C_{\text{bottom}} ) (cells/mL)</th>
<th>( C_{\text{total}} ) (cells/mL)</th>
<th>Viable cells (%)</th>
<th>HE (%)</th>
<th>( C_{\text{initial culture}} ) (cells/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.3</td>
<td>3.1</td>
<td></td>
<td>2.7\times10^6</td>
<td>1.3\times10^7</td>
<td>9.4\times10^7</td>
<td>31.4</td>
<td>35.3</td>
<td>3.0\times10^6</td>
</tr>
<tr>
<td>0.5</td>
<td>3.5</td>
<td>400</td>
<td>2.6\times10^6</td>
<td>1.3\times10^7</td>
<td>8.7\times10^7</td>
<td>29.1</td>
<td>40.0</td>
<td></td>
</tr>
<tr>
<td>0.7</td>
<td>4.1</td>
<td></td>
<td>2.2\times10^6</td>
<td>1.2\times10^7</td>
<td>7.3\times10^7</td>
<td>24.2</td>
<td>68.9</td>
<td></td>
</tr>
<tr>
<td>0.9</td>
<td>5.4</td>
<td></td>
<td>1.9\times10^6</td>
<td>1.2\times10^7</td>
<td>1.5\times10^7</td>
<td>4.8</td>
<td>52.8</td>
<td></td>
</tr>
</tbody>
</table>

It is possible to see in Table 12 that the viable cell concentration of the top layer decreased and the bottom remained practically the same with the increase of the current intensity.

Comparing the results present in Table 12 with Figures 33 to 36 (Appendix 2.7) is possible to see that for high current intensities a thickener mucous layer surrounding the microalgae flocs is formed. It was known that under some stress conditions, as high salinity and nutrient depletion, the palmelloid stage of the microalgae is induced. In this stage, non motile cells are surrounded in by a gelatinous mass of mucus that protect cells under stress conditions (Haydee Montoya & Alfred Olivera 1993; Oren 2005).

Comparing the results of the viable cell counting with the harvesting efficiency achieved (Figure 13), it was observed that for higher harvest efficiencies the viable cell concentration on the top increased while for the bottom decreased, due to the highest flocs accumulation on the top. However, is possible to see in Table 12 that for high harvesting efficiencies the cell concentrations on the top decreases while the bottom concentration remains practically the same. Possibly, the decrease of viable cell concentration on top is due to the increasing thickness of the mucus layer surrounding microalgae surface that can difficult the entrance of FDA in the cell, and consequently, the counting of viable cell concentration is affected. However, the use of FDA as the quenching of fluorescence during the counting assays can affect by itself the results, since the fluorochromes can have a different action depending on the used medium (Clarke et al. 2001; Pouneva 1997). Thus, probably there are more viable cells on the top for the highest harvesting efficiencies but the increasing current intensity acts as a stress condition which results in a thickener mucus layer.

The maintenance of viable cell concentration on the bottom when the electrical current was increased does not meet the expected result that would be a greater decrease of viable cells in the bottom when higher harvesting efficiency was achieved. Probably, this effect is due to a sampling error since when the samples were taken from the solution, microalgae
flocs were still coming to the surface and samples were not homogeneous. Thus, an over quantification may have been made, which is a weakness of the used methodology.

In general, the total viable cell concentration remains practically the same, except when current intensity of 0.9 A is applied, when the total viable cell concentration decreases more considerably, mainly due to the top viable cell concentration decreased.

It would be expected that, for a current intensity 0.9 A, the cells concentration on the bottom increases since the harvesting efficiency was lower. For this current intensity, it was verified a decrease of top viable cell concentration and no change on bottom viable cell concentration, even with the increase of OD₆₈₀, leading to the conclusion that for high current intensities the produced mucus may interfere with the FDA permeability in the cell, thereby affecting the counting, since higher viable cell concentration on the bottom would be expected. The FDA permeability can also be affected by the pH changes during the electrofloculation procedure, since the cell charge is affected (Lavoie et al. 2012). Another possible reason is due to the irreversible cell damage before this mucus surrounding layer was formed, causing the cell death.

To choose an optimum value of current intensity is necessary to take into account the influence of the microalgaes biomass properties in the β-carotene extraction procedure. Therefore, comparing the results of the harvesting efficiency and power consumption is not enough and is necessary to understand the membrane cell and environmental changes. Therefore, additional tests to better understand the influence of the mucus surrounding layer on Dunaliella salina β-carotene extraction procedure will be required.

![Figure 13. Results of the effect of the current intensity on cell viability: (a) optical density and (b) harvesting efficiency of Dunaliella salina orange culture with 120 g/L of NaCl.](image)

The data in Table 13 shows that both top and bottom viable cells concentration decreased with increasing of the stirring speed, for stirring velocities of 100 rpm up to 400 rpm. This can be explained by the mucus production that may interfere with the viable cell counting, as is
Results and Discussion

possible to see in Figures 38 to 44 (Appendix 2.7). However, this tendency was not verified for the assays without stirring and with stirring speed of 100 rpm, that can be explained by the lower stress that leads to a smaller mucus surrounding layer formation. At a stirring speed of 500 rpm the cells concentration decreased on the top layer and increased in the bottom when compared to the viable cell concentrations that was observed at 400 rpm. As already explained, at high stirring speeds the flocs are not strong enough to stay aggregated and the OD₄ of the medium increases. However the OD₄ for both stirring speeds, 400 rpm and 500 rpm, were similar and this difference in cell concentrations can be explained by sampling errors, as discussed above. At stirring speeds of 600 rpm, both top and bottom viable cell concentration decreased and OD₄ increased. Besides higher stirring speed can hamper flocs formation, in Figure 44 in Appendix 2.7, is possible to see that at 600 rpm the microalgae cells were destroyed, which cause the increase of the OD₄.

Table 13. Result of the cell counting varying the stirring speed.

<table>
<thead>
<tr>
<th>I (A)</th>
<th>V (volt)</th>
<th>Stirring speed (rpm)</th>
<th>C_top (cells/mL)</th>
<th>C_bottom (cells/mL)</th>
<th>C_total (cells/mL)</th>
<th>Viable cells (%)</th>
<th>HE (%)</th>
<th>C_initial culture (cells/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.8</td>
<td>0</td>
<td>1.3×10⁶</td>
<td>1.5×10⁶</td>
<td>1.4×10⁶</td>
<td>46.8</td>
<td>47.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.8</td>
<td>100</td>
<td>2.1×10⁶</td>
<td>6.3×10⁵</td>
<td>1.0×10⁶</td>
<td>34.8</td>
<td>66.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.7</td>
<td>200</td>
<td>1.9×10⁶</td>
<td>4.5×10⁵</td>
<td>8.6×10⁵</td>
<td>28.7</td>
<td>65.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.7</td>
<td>3.4</td>
<td>1.7×10⁶</td>
<td>3.1×10⁵</td>
<td>6.8×10⁵</td>
<td>22.6</td>
<td>77.3</td>
<td>3.0×10⁶</td>
<td></td>
</tr>
<tr>
<td>3.4</td>
<td>400</td>
<td>1.6×10⁶</td>
<td>2.4×10⁵</td>
<td>6.4×10⁵</td>
<td>21.2</td>
<td>95.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.6</td>
<td>500</td>
<td>8.7×10⁵</td>
<td>6.5×10⁵</td>
<td>4.3×10⁵</td>
<td>14.3</td>
<td>90.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.6</td>
<td>600</td>
<td>2.7×10⁵</td>
<td>1.2×10⁵</td>
<td>1.6×10⁵</td>
<td>5.4</td>
<td>13.6</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Comparing the results present in Table 12 with Figures 38 to 44 is possible to see that for high stirring speeds there is a formation of a more predominant mucous layer surrounding the microalgae flocs, which can interfere with the FDA permeability in the cell and affect the viable cell counting.

Comparing the harvesting efficiency results achieved in the present test with the others tests in the same conditions, i.e. stirring speed of 400 rpm and a current intensity of 0.7 A, it is clearly that in the present test the harvesting efficiency achieved was higher, having reached 95.6%, and the power consumption was lower, it was about 0.34 kWh/m³, since lower voltage was required. This can be due to the lower culture car/chl ratio (car/chl=4.3) in comparison with the other cultures (higher than 5). Low car/chl ratio means the culture are in an intermediate phase and, because of that, the membrane characteristics may have changed. Another reason can be due to the fact that in this assay the equipment used to measure the OD
was different, due to their unavailability. A harvesting efficiency of 94% was achieved using similar electroflocculation conditions and using the *Dunaliella salina* green culture (Mohammad-Ghasemnejadmaleki et al. 2014; Zenouzi et al. 2013). Thus, it will be necessary additional studies to better understand the effect of the microalgae phase on harvesting efficiency.

In conclusion, the methodology of viable cell counting by FDA appears to have some disadvantages, like difficult sampling, time consuming, requires equipment such as fluorescence microscopy and fluorescence techniques and is difficult to close the balance due to cell damage. The fact that whole cells that do not stain with FDA may be explained by the mucus layer formation under stress conditions which may hinder the cell extraction process and thereby a depreciation of the product, i.e, β-carotene.

In choosing an optimum value of stirring speed and current intensity is necessary to take into account the influence of the microalgae biomass properties in the β-carotene extraction procedure. Thereby, comparing the results of the harvesting efficiency and power consumption is not enough and will be required additional tests to better understand the influence of each parameter, during the optimization, in the membrane cell and environmental changes to optimize the extraction procedure too. Nowadays, there are several methods used to extract the carotenoids content from microalgae slurry or dry powder, which include extraction with conventional organic solvents (e.g., hexane and ethanol), supercritical CO₂ or using an edible oil (Hosseini Tafreshi & Shariati 2009).

During the assays the electrodes mass was monitored and the results are present in Table 14. The mass of the anode during the assays remained, practically, the same. However, the mass of the cathode increased during the assays. After some experimental procedures, there
appears a white layer above the cathode (fouling) that was removed using a nitric acid 0.5 M solution. The first time the cathode was cleaned the mass changes was more noticeable. After that, the mass remained, practically, constant and equal to the initial mass.

During all assays, about 133, the mass of the anode decrease, approximately, 25 mg, which corresponds to a loss of 0.11%. Similar studies with aluminum electrodes, for 0.3 A, 228 rpm, electrode gap of 1.29 cm and an electrode surface area of 25 cm², revealed that, for the same electroflocculation time, an electrode dissolution of 47 mg per liter of culture treated was observed. In the present study 67 L of culture was treated during approximately 13.3 h, would result in 3.1 g of an aluminum electrode dissolution, approximately. Thus, for large scale applications, the use of non sacrificial electrodes will turns out to be very important (Mohammad-Ghasemnejadmaleki et al. 2014).

Table 14. Electrodes mass during the experimental procedure.

<table>
<thead>
<tr>
<th>Date</th>
<th>Electrode mass (anode) (g)</th>
<th>Electrode mass (cathode) (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>21-04-2016 (initial)</td>
<td>22,1667</td>
<td>22,2895</td>
</tr>
<tr>
<td>23-04-2016 (after 11 repetitions)</td>
<td>22,1487</td>
<td>22,3838</td>
</tr>
<tr>
<td>9-05-2016 (after 14 repetitions)</td>
<td>22,1354</td>
<td>22,4919</td>
</tr>
<tr>
<td>10-05-2016 (after 12 repetitions)</td>
<td>22,1328</td>
<td>22,5114</td>
</tr>
<tr>
<td>17-05-2016 (after 17 repetitions)</td>
<td>22,1356</td>
<td>22,5209</td>
</tr>
<tr>
<td>18-05-2016 (after 12 repetitions)</td>
<td>22,1388</td>
<td>22,5372</td>
</tr>
<tr>
<td>19-05-2016 (after 11 repetitions)</td>
<td>22,1340</td>
<td>22,5604</td>
</tr>
<tr>
<td>31-05-2016 (after 11 repetitions)</td>
<td>22,1333</td>
<td>22,2658*</td>
</tr>
<tr>
<td>1-06-2016 (after 12 repetitions)</td>
<td>22,1340</td>
<td>22,2649*</td>
</tr>
<tr>
<td>20-06-2016 (after 33 repetitions)</td>
<td>22,1344</td>
<td>22,2646*</td>
</tr>
</tbody>
</table>

* after cathode cleaning with a solution of Nitric acid 0.5 M.
Chapter 5: Conclusions

Comparing the existing technologies for *Dunaliella salina* harvesting, the results obtained in this study suggest that electroflocculation has a high potential and is a promising harvesting technique, due to the absence of added chemicals, low maintenance costs and the harvesting can be done continuously. The use of non-sacrificial electrodes reduces operating costs and environmental contamination due to insignificant mass loss during the assays.

The influence of the different variables on the electroflocculation was studied using a one-variable-at-a-time approach. Successively, the influence of the electrode distance, electroflocculation time, stirring speed, current intensity and electrical conductivity was investigated. The highest harvesting efficiency and lower power consumption were achieved with 1 cm of electrode distance and 6 min of electroflocculation time with stirring at 400 rpm and current intensity of 0.7 A. Using the optimized parameters in the lab-scale electroflocculation unit a harvesting efficiency of 71.1% with a power consumption of 0.63 KWh/m$^3$ was achieved.

The study of the effect of the electrical conductivity on power consumption reveals that the energy consumption is lower for solutions with high salt concentration, as saltwater, than for low salt solutions, as freshwater, which is an advantage for *Dunaliella salina* harvesting.

Cell viability was studied by microscopy using FDA as fluorochrome. In this study, the cell viability was used as a complementary methodology and it was found that the viability decreases with the increase of both current intensity and stirring speed. For the optimized parameters cell viability was 21.2%. Cell viability shall be used as a complementary methodology when electroflocculation parameters are optimized. However, cell viability methodology needs to be improved.

In conclusion, this study provides a good base for future tests, having already defined a good evaluation methodology for a given set of parameters. However, it needs significant research from the economical point of view but also on cell viability.
Chapter 6: Further research and Final appreciation

6.1 Further research

It is known that microalgae harvesting systems can be very complex, involving numerous parameters interactions that depend of the culture medium and environment conditions. Therefore, further research is required to better understand these interactions.

Future work on charge changes during electroflocculation should be conducted to better understand the electroflocculation process and minimize harvesting time, which can be studied based on zeta potential.

The effect of cell concentration and pH on harvesting efficiency and power consumption needs to be further investigated. It is also necessary to evaluate the influence of different non sacrificial electrode materials, as platinum and titanium, on electroflocculation efficiency and power consumption.

In this study was demonstrated the technical viability of the electroflocculation; however, the study of cell viability after this procedure needs to be better examined. The viability can be studied by flow citometry, in which cells are detectable with or without fluorescent staining. In this technique can be studied the used of FDA, PI and others fluorochromes, since different fluorochromes have different behaviors. However, it needs to be study the influence of the medium used and cell membrane properties in cell viability measurement. Cell viability needs also to be studied in each optimization step to guarantee the best result associated with the desired cells membrane properties.

In conclusion, further tests are needed to determine the economic viability and scale-up of this novel and promising microalgae harvesting method.

6.2 Final appreciation

This thesis allowed me to take a decision about one of the most viable and promising harvesting method to study and also to learn about different areas of my interest, which enabled, on the whole, a more careful attitude towards each stage of this work.

The main purpose of this project, which was to develop a method for harvesting Dunaliella salina with high efficiency and low power requirement, was achieved. Beyond the study of several electroflocculation parameters, a first approach to cell viability was taken, however, the methodology needs to be optimized.
In conclusion, the results obtained in the present study clearly demonstrate that a promising harvesting method has been developed with high potential for being used in microalgae applications.
References

A4F Algae For future, Available at: http://www.a4f.pt/ [Accessed May 5, 2016].
Evaluation of electroflocculation for harvesting Dunaliella salina using non sacrificial electrodes


Evaluation of electrofloculation for harvesting Dunaliella salina using non sacrificial electrodes


LiqTech. *Pre-concentration of algae filtration of Dunaliella algae with Sic ceramic membranes*. Available at: http://www.liqtech.dk/img/user/file/Case_story_algae_pre_concentration_v2.pdf
Evaluation of electrofloculation for harvesting Dunaliella salina using non sacrificial electrodes

References


Evaluation of electrofloculation for harvesting Dunaliella salina using non sacrificial electrodes


June 13, 2016].


Thick, P. et al., 2016. A use of microbubbles continued flotation tank to collect the algal cells runway means, CN Patent 204981856 U.


Wiemers, R.A. & Kohlheb, R., 2014. Non-sacrificial electrodes and/or coils for immersed
Evaluation of electroflocculation for harvesting Dunaliella salina using non sacrificial electrodes
Appendix 1. Available patents of microalgae harvesting techniques

<table>
<thead>
<tr>
<th>Title</th>
<th>Publication date</th>
<th>Patent number</th>
<th>Applicant</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>A use of microbubbles continued flotation tank to collect the algal</td>
<td>2016</td>
<td>CN 204981856 U</td>
<td>Ocean University of China</td>
<td>The present invention provides a continuous micro-bubble flotation method to collect the runway pool algal cells.</td>
</tr>
<tr>
<td>cells runway means (Thick et al. 2016).</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adsorptive bubble separation methods and systems for dewatering</td>
<td>1999</td>
<td>US 5951875 A</td>
<td>Eastman Chemical Company</td>
<td>The present invention includes an adsorptive bubble separation method that is capable of dewatering the algae, achieving algae concentrations from which extractable components can be economically removed. In this technique chemical additives and treatments can be avoided and both growth medium and algae residue can be returned to the source after components extraction, if desired.</td>
</tr>
<tr>
<td>suspensions of microalgae and extracting components therefrom (Kanel</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&amp; Guelcher 1999).</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Algal cell harvesting (Kessler 1992).</td>
<td>1982</td>
<td>US 4324067 A</td>
<td>The University Of Arizona</td>
<td>The present invention relates to a system, process and apparatus for harvesting swimming unicellular algae. The invention relates to a system for producing accelerated migration of unicellular motile microalgae cells from a reservoir in which cells are disposed for multiplication by cell division to a cell harvesting zone located adjacent the top surface of the reservoir. Mass of discrete elements connecting with the harvesting zone provides a migration path which enables cells migration to the harvesting zone.</td>
</tr>
<tr>
<td>Foundation</td>
<td></td>
<td></td>
<td>Foundation</td>
<td></td>
</tr>
<tr>
<td>Apparatus for electrodewatering (Miller et al. 2005).</td>
<td>2005</td>
<td>US 6871744 B2</td>
<td>Crc For Waste Management &amp;</td>
<td>The present invention provides an apparatus for electrodewatering and recovery of a solid-liquid mixture. The apparatus have a conveyor belt adapted for receiving the solid-liquid mixture thereon and for receiving an electric charge that helps the separation.</td>
</tr>
<tr>
<td>Pollution Control Limited</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
### Table 15. Available patents of microalgae harvesting techniques (continuation).

<table>
<thead>
<tr>
<th>Title</th>
<th>Publication date</th>
<th>Patent number</th>
<th>Applicant</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dunaliella collection method (Feng et al. 2015).</td>
<td>2015</td>
<td>CN 104726340 A</td>
<td>Ding Feng River</td>
<td>In this method <em>Dunaliella</em> culture is pumped into an upper cylinder via a feed port, then is added a flocculant, PFS that is non-toxic and harmless inorganic polymers and the solution is mixed. After this step, <em>Dunaliella salina slurry</em> is completely separated from the culture medium, by opening the discharge port valve, and is washed in order to achieve high purities.</td>
</tr>
<tr>
<td>Electrolytic system and method for filtering an aqueous particulate suspension (Taylor et al. 2012).</td>
<td>2012</td>
<td>US 20120091000 A1</td>
<td>Faraday Technology, Inc.</td>
<td>The present invention provides an electrolytic filtration method and apparatus for the concentration and collection of suspended particulates in a solution. The electrolytic cell contains at least an anode and a cathode. The electrolytic cell also contains a filter, and in one embodiment the filter is a moving belt filter. The electrolytic filtration method and apparatus is based on the electrophoretic movement of microalgae particles suspended in an aqueous solution caused by the existence of an electric field.</td>
</tr>
<tr>
<td>Electromembrane apparatus and process for preventing membrane fouling (Muralidhara 1991).</td>
<td>1991</td>
<td>US 5043048 A</td>
<td>Muralidhara Harapanahalli S</td>
<td>The present invention provides a crossflow apparatus having an electrofilter disposed therein. The continuously separation method reduce the membrane or filter fouling. The aqueous suspension is pumped into the crossflow apparatus at a pressure that promotes the flow of filtrate through the electrofilter and, at the same time, supplies electrical energy to the electrofilter at a voltage adapted to reduce the fouling.</td>
</tr>
<tr>
<td>Electro-penetrative type sludge weight reducing apparatus (Song &amp; Woo 2012).</td>
<td>2012</td>
<td>US 20120091006 A1</td>
<td>Dong-Ill Canvas Engineering Co., Ltd</td>
<td>The present invention provides an electro-penetrative sludge weight reducing apparatus (belt filter), which can uniformly and efficiently dehydrate the sludge by pressurizing the entire surface of the sludge with the same pressure and applying the same voltage.</td>
</tr>
</tbody>
</table>
Table 15. Available patents of microalgae harvesting techniques (continuation).

<table>
<thead>
<tr>
<th>Title</th>
<th>Publication date</th>
<th>Patent number</th>
<th>Applicant</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fouling control in membrane filtration processes (Ivo Vankelecom n.d.).</td>
<td>2012</td>
<td>WO 2012083390 A2</td>
<td>Katholieke Universiteit Leuven</td>
<td>The present invention provides a tunable magnetically induced vibration system for fouling control in membrane filtration processes. A variable magnetic flux acts directly or indirectly on a (magnetic) membrane inducing its vibration. The vibration enhances shear-rate of the fluid to be separated near the membrane surface to control fouling in-situ.</td>
</tr>
<tr>
<td>Functionalized ceramic membranes for the separation of organics from raw water and methods of filtration using functionalized ceramic membranes (Votaw et al. 2014).</td>
<td>2014</td>
<td>WO 2014081737 A1</td>
<td>Lance Energy Services, L.L.C.</td>
<td>The present invention involves using silicon carbide membranes fabricated (sometimes hereafter - &quot;SiC&quot;). SiC-based membranes have been studied in some detail with respect to their inherent hydrophobicity and hydrophilicity. Si-terminated surfaces tend to be hydrophilic, and C-terminated surfaces are more hydrophobic.</td>
</tr>
<tr>
<td>Harvesting algae from water (Langer et al. 2015).</td>
<td>2015</td>
<td>US 2015284673 A1</td>
<td>Sapphire Energy, Inc</td>
<td>The present invention involves a method for harvesting microalgae from saline water using polymer flocculants with or without a dissolved air flotation.</td>
</tr>
<tr>
<td>High-volume fast separation of multi-phase components in fluid suspensions (Dionne et al. 2012).</td>
<td>2012</td>
<td>US 20120325727 A1</td>
<td>Flodesign Sonics, Inc</td>
<td>The present invention utilizes acoustophoresis (which means migration with sound.), a low-power, no-pressure-drop, no-clog, solid-state approach to particle removal from fluid suspensions. It is used to achieve separations that are more typically performed with porous filters and centrifuges, but it has none of the disadvantages of these systems.</td>
</tr>
</tbody>
</table>
### Table 15. Available patents of microalgae harvesting techniques (continuation).

<table>
<thead>
<tr>
<th>Title</th>
<th>Publication date</th>
<th>Patent number</th>
<th>Applicant</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Method and apparatus for continuous flow membrane-less algae dewatering (Lean et al. 2010).</td>
<td>2010</td>
<td>US 20100314323 A1</td>
<td>Palo Alto Research Center Incorporated</td>
<td>In the present invention a system comprises an inlet to receive at least a portion of the fluid containing microalgae; a curved channel within which the fluid containing microalgae flows in a manner such that the neutrally buoyant microalgae flow in a band offset from a center of the curved channel; a first outlet for the fluid with algae; and a second outlet for the remaining fluid.</td>
</tr>
<tr>
<td>Method for harvesting algae (Curtain &amp; Snook 1985).</td>
<td>1985</td>
<td>US 4554390 A</td>
<td>Commonwealth Scientific And Industrial Research Organization, Betatene Limited</td>
<td>The present invention provides a method for harvesting <em>Dunaliella salina</em> from brines suspensions containing sodium chloride at a concentration of about 3M or above, wherein the algal suspension is contacted with an adsorbent having a hydrophobic surface so as to adsorb the algae thereon, and then is separated from the brine suspension.</td>
</tr>
<tr>
<td>Method for increasing the potential for biofuel production from microalgae by using bio-modulators (Wahby et al. 2014).</td>
<td>2014</td>
<td>WO 2014003530 A4</td>
<td>Moroccan Foundation For Advanced Science.</td>
<td>The present invention relates to an application of ad hoc changes to the pH of the cultivation media causing the spontaneous precipitation of cells loaded with lipids. The recovery phase of the biomass occurs by a change in the pH of the culture medium with an addition of NaOH or KOH (bio-modulators III) to obtain pH 10.</td>
</tr>
<tr>
<td>Method of <em>Dunaliella</em> collecting and beta-carotene extracting (Yuan et al. 1994).</td>
<td>1994</td>
<td>CN 1084848 A</td>
<td>Nanjing Normal University</td>
<td>The present invention relates to a dilution of salt algae solution with water to lower the density of the solution, and the salt algae are collected by stagnant sedimentation or centrifugal sedimentation. β-carotene is extracted with vegetable oil or organic solvents.</td>
</tr>
</tbody>
</table>
Table 15. Available patents of microalgae harvesting techniques (continuation).

<table>
<thead>
<tr>
<th>Title</th>
<th>Publication date</th>
<th>Patent number</th>
<th>Applicant</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Method of growing and harvesting microorganisms (Algae Farms 1990)</td>
<td>1990</td>
<td>US 4958460 A</td>
<td>Algae Farms</td>
<td>The present invention relates to a system for growing and harvesting <em>Dunaliella salina</em> along with their associated bacteria. The algae are grown in saline water and form flocs, associated with bacteria, in the upper regions of the body of water by the action of light. The flocs are harvested off the surface by skimming or other means.</td>
</tr>
<tr>
<td>Methods for concentrating microalgae (Radaelli <em>et al.</em> 2009).</td>
<td>2009</td>
<td>US 20090162919 A1</td>
<td>Aurora Biofuels, Inc.</td>
<td>The present invention provides an economical and industrial-scale method for the flocculation of microalgae that do not form flocs spontaneously (e.g., microalgae with an average diameter of about 10 μm or less,) using low concentrations of organic flocculants (e.g., less than 10% of the dry weight of biomass).</td>
</tr>
<tr>
<td>Microalgae centrifugal filtration device (Lin 2014).</td>
<td>2014</td>
<td>CN 203803660 U</td>
<td>Tianyi Lin</td>
<td>The present invention provides a centrifugal filtration device for microalgae harvesting.</td>
</tr>
<tr>
<td>Microalgae integration device (Fengjie <em>et al.</em> 2014).</td>
<td>2014</td>
<td>CN 203878147 U</td>
<td>Dafeng City, Jia Feng Oil Co</td>
<td>The present invention is intended to provide a microalgae culture, harvesting and drying integrated device. The microalgae collection doesn’t need the addiction of flocculants or centrifugation. The apparatus of the present invention includes a membrane system (that has an inclination), the shaft means, conveyor systems and drying equipment.</td>
</tr>
</tbody>
</table>
Table 15. Available patents of microalgae harvesting techniques (continuation).

<table>
<thead>
<tr>
<th>Title</th>
<th>Publication date</th>
<th>Patent number</th>
<th>Applicant</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microalgae separator apparatus and method (Borodyanski &amp; Konstantinov 2003).</td>
<td>2003</td>
<td>US 6524486 B2</td>
<td>Sepal Technologies Ltd.</td>
<td>The present invention provides a method that comprises the steps of flocculation, flotation and dehydration. Microalgae suspension is passed to a mixer unit where flocculation is carried out. The suspension is then directed to a flotation column where dissolved gases pass through a disperser. A layer of microalgae based foam is formed on the top of the column, which can be skimmed off followed by dehydration step.</td>
</tr>
<tr>
<td>Microbubble generating device and contaminated water purifying system provided with microbubble generating device (Tamura &amp; Kazuyoshi Adachi 2015).</td>
<td>2015</td>
<td>WO 2015060382 A1</td>
<td>Earth Re-Pure Co., Ltd</td>
<td>The present invention relates to a water purification system comprising a micro-bubble generating device. Microbubble generating device is provided with a liquid inflow channel, a discharge channel, a middle part that connects the liquid inflow channel and the discharge channel and a gas supply channel.</td>
</tr>
<tr>
<td>Microalgae harvest module and method of harvesting microalgae (Lin et al. 2015).</td>
<td>2015</td>
<td>TW 201525130 A</td>
<td>National Chiao Tung University</td>
<td>The present invention provides a method where flocculants and magnetic particles are added into a medium containing microalgae, so as to form microalgae magnetic flocs. A magnetic field is applied to an outer wall of the harvesting container, and thus the microalgae magnetic flocs are adhered to an inner wall of the harvesting container. The magnetic field is removed to obtain the microalgae magnetic flocs.</td>
</tr>
<tr>
<td>Process and apparatus for treating sludge (Berrak &amp; Dermoune 2005).</td>
<td>2005</td>
<td>US 20050199499 A1</td>
<td>Les Technologies Elcotech Inc.</td>
<td>The present invention relates to an apparatus for treating sludge comprising electrodes including at least one anode and at least one cathode. The electrodes define between them a space where the sludge is submitted an electric current, thereby occurs dehydrating of the sludge and is generated an effluent.</td>
</tr>
</tbody>
</table>
### Table 15. Available patents of microalgae harvesting techniques (continuation).

<table>
<thead>
<tr>
<th>Title</th>
<th>Publication date</th>
<th>Patent number</th>
<th>Applicant</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Process for microalgae conditioning and concentration (Clayton et al. 2013)</td>
<td>2013</td>
<td>US 20130344575 A1</td>
<td>Renewable Algal Energy, Llc.</td>
<td>In this invention the conditioning and concentration of microalgae are accomplished by the process steps of grinding a dilute aqueous dispersion of microalgae in the presence of a grinding media and then applying adsorptive bubble separation.</td>
</tr>
<tr>
<td>System and method for high-voltage pulse assisted aggregation of algae (Schafran et al. 2014)</td>
<td>2014</td>
<td>US 8772004 B2</td>
<td>Old Dominion U.R.F.</td>
<td>The present invention provides an microalgae aggregation by applying a nanosecond pulsed electric field, which can include a plurality of electric pulses having a pulse duration ranging from 1 to 1,000 nanoseconds.</td>
</tr>
<tr>
<td>Systems, methods and apparatuses for dewatering, flocculating and harvesting algae cells (Green et al. 2012)</td>
<td>2012</td>
<td>US 20120129244 A1</td>
<td>Michael Phillip Green</td>
<td>The process includes dewatering and harvesting method for microalgae using an electromotive force. An electromagnetic field is applied to an aqueous suspension of microorganisms causing their flocculation, which are harvested by skimming.</td>
</tr>
<tr>
<td>Vibrationally-induced dynamic membrane filtration (DiLeo et al. 1999)</td>
<td>1999</td>
<td>US 5985160 A</td>
<td>Millipore Corporation</td>
<td>The present invention provides a filtration channel comprised of a stationary membrane disc mounted in close proximity to a solid disc capable of being rotated at high angular velocities. The mechanical rotation of the solid disc causes the fluid flow, between the membrane and solid disc, in a direction generally parallel to the membrane surface, thereby promoting the concentration and minimizing the fouling.</td>
</tr>
</tbody>
</table>
Appendix 2. Culture and microscope images

2.1 Preliminary tests

Figure 15. Result of the preliminary tests using the (a) initial Dunaliella salina brown culture with 120 g/L of NaCl, (b) with no stirring and with (c) stirring.

2.2 Influence of the electrode distance on electroflocculation

Figure 16. Microscope observation (enlargement 40x) of Dunaliella salina orange culture with 120 g/L of NaCl before the electroflocculation.

Figure 17. Results of the effect of the electrode distance on electroflocculation: b1 (1cm), b2 (2 cm), b3 (3cm), b4 (4 cm) and b5 (5 cm).
2.3 Influence of the time on electroflocculation

**Figure 18.** Results of the effect of the time on electroflocculation: c1 (1 min), c2 (2 min), c3 (4 min), c4 (6 min), c5 (8 min) and c6 (10 min).

**Figure 19.** Microscope observation (enlargement 40x) of the cultures, after the electroflocculation, that achieved higher harvest efficiencies: c4 (6 min), c5 (8 min) and c6 (10 min).
2.4 Influence of the stirring speed on electroflocculation

![Figure 20](image1.png)

**Figure 20.** Results of the effect of the stirring speed on electroflocculation: d1 (0 rpm), d2 (100 rpm), d3 (200 rpm), d4 (300 rpm), d5 (400 rpm), d6 (500 rpm) and d7 (600 rpm).

![Figure 21](image2.png)

**Figure 21.** Microscope observation (enlargement 40x) of the cultures after electroflocculation that achieved higher harvest efficiencies: d2 (100 rpm), d3 (200 rpm), d4 (300 rpm) and d5 (400 rpm).

2.5 Influence of the current intensity on electroflocculation

![Figure 22](image3.png)

**Figure 22** Results of the effect of the current intensity on electroflocculation: e1 (0.3 A), e2 (0.5 A), e3 (0.7 A) and e4 (0.9 A).
Evaluation of electroflocculation for harvesting *Dunaliella salina* using non sacrificial electrodes

**Figure 23.** Result of the test using water applying 0.9 A.

**Figure 24.** Microscope observation (enlargement 40x) of e1 (0.3 A), e2 (0.5 A), e3 (0.7 A) and e4 (0.9 A)

2.6 Influence of the electrical conductivity on electroflocculation

10^2 g/L NaCl

**Figure 25.** Results of the effect of the EC on electroflocculation using a *Dunaliella salina* culture with 10^2 g/L of NaCl: f1_{102} (0.3 A); f2_{102} (0.5 A); f3_{102}(0.7 A) and f4_{102}(0.9 A).
Evaluation of electroflocculation for harvesting *Dunaliella salina* using non sacrificial electrodes

**Figure 26.** Microscope observation (enlargement 40x) after electroflocculation of f3\textsubscript{120} (0.7 A).

120 g/L NaCl

**Figure 27.** Results of the effect of the EC on electroflocculation using a *Dunaliella salina* culture with 120 g/L of NaCl: f1\textsubscript{120} (0.3 A); f2\textsubscript{120} (0.5 A); f3\textsubscript{120} (0.7 A) and f4\textsubscript{120} (0.9 A).

**Figure 28.** Microscope observation (enlargement 40x) after electroflocculation of f3 (120) (0.7 A).
Evaluation of electroflocculation for harvesting *Dunaliella salina* using non sacrificial electrodes

146 g/L NaCl

**Figure 29.** Results of the effect of the EC on electroflocculation using a *Dunaliella salina* culture with 146 g/L of NaCl: f1\textsubscript{146} (0.3 A); f2\textsubscript{146} (0.5 A), f3\textsubscript{146} (0.7 A) and f4\textsubscript{146} (0.9 A).

**Figure 30.** Microscope observation (enlargement 40x) after electroflocculation of f3 (146) (0.7 A).

2.7 Measurement of cell viability

**Figure 31.** Results of the effect of the current intensity on cell viability: h1 (0.3 A); h2 (0.5 A), h3 (0.7 A) and h4 (0.9 A).
Figure 32. Example of a green and red cell observed in a sample during cell counting with FDA.

Figure 33. Microscope observation (enlargement 40x) after electroflocculation of h1 (0.3 A) on the bottom (left) and on top (right).

Figure 34. Microscope observation (enlargement 40x) after electroflocculation of h2 (0.5 A) on the bottom (left) and on top (right).
Evaluation of electroflocculation for harvesting *Dunaliella salina* using non sacrificial electrodes

Figure 35. Microscope observation (enlargement 40x) after electroflocculation of h4 (0.9 A) on the bottom (left) and on top (right).

Figure 36. Microscope observation (enlargement 40x) after electroflocculation of h3 (0.7 A) on the bottom (left) and on top (right).

Figure 37. Results of the effect of the stirring speed on cell viability: h1 (0 rpm), h2 (100 rpm), h3 (200 rpm), h4 (300 rpm), h5 (400 rpm), h6 (500 rpm) and h7 (600 rpm).
Figure 38. Microscope observation (enlargement 40x) after electrofloculation of h1 (0 rpm) on the bottom (left) and on top (right).

Figure 39. Microscope observation (enlargement 40x) after electrofloculation of h2 (100 rpm) on the bottom (left) and on top (right).

Figure 40. Microscope observation (enlargement 40x) after electrofloculation of h3 (200 rpm) on the bottom (left) and on top (right).
Evaluation of electroflocculation for harvesting *Dunaliella salina* using non sacrificial electrodes

**Figure 43.** Microscope observation (enlargement 40x) after electroflocculation of h4 (300 rpm) on the bottom (left) and on top (right).

**Figure 42.** Microscope observation (enlargement 40x) after electroflocculation of h5 (400 rpm) on the bottom (left) and on top (right).

**Figure 41.** Microscope observation (enlargement 40x) after electroflocculation of h6 (500 rpm) on the bottom (left) and on top (right).
**Figure 44.** Microscope observation (enlargement 40x) after electroflocculation of h7 (600 rpm) on the bottom (left) and on top (right).