Intensive production of microalgae with high economic, social and energetic value: potential in the Autonomous Region of the Azores

Tese de Doutoramento

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À minha mulher e bebé

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RESUMO

As microalgas são fonte de compostos de elevado valor comercial como sejam os ácidos gordos polinsaturados e os carotenoides, produtos com interesse crescente a nível biotecnológico devido aos benefícios que representam para a saúde humana. Atualmente apenas algumas espécies de microalgas estão bem estabelecidas no mercado, havendo a necessidade de encontrar espécies indígenas com potencial biotecnológico, uma vez que estas estão adaptadas às condições bióticas e abióticas prevalecentes em cada região, sendo assim bons candidatos à produção de recursos de origem biológica.

No presente estudo, o potencial dos Açores para a produção de microalgas no exterior foi avaliado. Analisaram-se fatores como água, luz, temperatura, fontes de carbono, nutrientes, conjuntura internacional e contexto regional. A análise SWOT resultante indicou que o arquipélago é um local promissor para implementar um projeto comercial de cultivo ao ar livre de microalgas tendo em vista a produção de produtos de valor acrescentado.

A partir de amostras coletadas em 23 locais da ilha de São Miguel, Açores obtiveram-se 114 isolados de microalgas. Os isolados, pertencendo a 60 *taxa*, compreendiam 39 Chlorophyta, 10 Ochrophyta, 6 Cyanophyta, 3 Charophyta, 1 Euglenozoa e 1 Cryptophyta. Dezoito desses *taxa* constituem novos registos para a ilha de São Miguel. Cinco dos isolados foram selecionados para investigação posterior nomeadamente no que concerne ao potencial de cultivo e produção de ácidos gordos polinsaturados e os carotenoides.

As cinco estirpes isoladas de microalgas foram cultivadas no exterior em fotobiorreactores cilíndricos verticais para avaliar o respetivo potencial de crescimento e analisar a composição da biomassa produzida em termos de ácidos gordos e carotenóides secundários. Como seria de esperar, o verão foi a estação mais produtiva para todos os parâmetros avaliados. *Chlorococcum* sp. foi a estirpe mais interessante devido à sua elevada produção de ácidos gordos insaturados e carotenóides secundários. Os valores obtidos revelaram-se interessantes quando comparados com outros obtidos para estirpes utilizadas comercialmente. Isto indica que a prospeção e seleção de estirpes indígenas é um meio eficiente de descobrir microalgas com aplicações industriais passíveis de serem alvo de cultivo no exterior.

O efeito da concentração de nutrientes (com ênfase no bicarbonato) sobre o crescimento e acumulação de carotenóides da estirpe indígena de *Chlorococcum*. foi testado em cultivo exterior em fotobiorreactores cilíndricos de coluna de bolhas. Os resultados obtidos demonstraram que a estirpe cresceu bem, apresentando um crescimento máximo comparável ou mesmo mais elevado do que aquele reportado para condições otimizadas de cultivo. Demonstraram igualmente que o uso de bicarbonato como fonte de carbono aumenta a produção de carotenoides secundários pela estirpe em estudo.

Em resumo, a estirpe indígena de *Chlorococcum* demonstrou ser um bom candidato para uma elevada produção de carotenóides secundários e ácidos gordos em cultivos no exterior. No entanto, são necessários estudos complementares envolvendo a otimização do cultivo e a indução de stress, para elevar o processo de produção à escala industrial.

Em geral, os resultados sugerem que os Açores têm condições para implementar uma produção de produtos de valor acrescentado a partir de microalgas recorrendo a cultivo no exterior, proporcionando benefícios significativos a partir de um recurso económico alternativo.

Palavras-chave: prospeção de estirpes indígenas, cultivo no exterior, bicarbonato como fonte de carbono, Açores, carotenóides secundários, ácidos gordos, *Chlorococcum* sp.

ABSTRACT

Microalgae are a promising source of high-value compounds such as polyunsaturated fatty acids and carotenoids, products with great commercial interest in biotechnology due to their association with various health benefits. Currently only a few species of microalgae are well established in the market and there is the need to find promising indigenous microalgae species with biotechnological potential, since these are adapted to the prevailing regional abiotic and biotic factors and are, therefore, good candidates for bioresource production.

In this study the potential of the Azores for outdoor microalgae production was evaluated. Factors such as water, light, temperature, carbon sources, nutrients, international outlook and regional context were analysed. The resulting SWOT analysis indicated that the Azores is a promising location to implement a commercial outdoor production of value-added products from microalgae.

Following a survey in 23 locations of the island of São Miguel, Azores, 114 microalgae isolates were obtained. From them, 60 taxa were identified, comprising 39 Chlorophyta, 10 Ochrophyta, 6 Cyanophyta, 3 Charophyta, 1 Euglenozoa and 1 Cryptophyta. Eighteen of the taxa identified constitute new records for the island. Five isolates were selected for further research covering culture studies and fatty acid and secondary carotenoids production.

The five selected isolated strains of microalgae were cultivated outdoors in cylindrical vertical photobioreactors to evaluate their growth potential and analyse the composition of the biomass produced in terms of fatty acids and secondary carotenoids. As expected, summer was the most productive season for all the evaluated parameters. *Chlorococcum* sp. was the most interesting strain due to its high production of both unsaturated fatty acids and secondary carotenoids. The values obtained compared favourably with those of commercially used strains, indicating that prospection and screening of indigenous strains for outdoor production has good potential to detect microalgae with industrial applications.

The effect of nutrient concentrations (with emphasis on bicarbonate) on the growth and carotenoid accumulation of the indigenous strain of *Chlorococcum* sp. was tested in outdoor batch cultivation, carried out in tubular bubble column photobioreactors. The results obtained demonstrated that the strain grew well, with a maximum specific growth comparable or higher than those reported in optimized indoor conditions. Results further show that using bicarbonate as a carbon source is a way to increase the commercial outdoor production of secondary carotenoids in the studied indigenous *Chlorococcum* strain.

In summary, the indigenous *Chlorococcum* was seen to be a good target for the production of secondary carotenoids and fatty acids in a commercial outdoor production. Nevertheless, further studies encompassing culture optimization, including stress induction, are needed for a scale up of the industrial production process.

Overall, results suggest that the Azores has conditions to implement outdoor production of value-added products from microalgae, providing end-users relevant benefits from an alternative economic resource.

Keywords: prospection of indigenous strains, outdoor production, bicarbonate as carbon source, Azores, secondary carotenoids, fatty acids, *Chlorococcum* sp.

CHAPTER 1. INTRODUCTION

Pormenor do arejamento da cultura
(foto de Xavier E)

CHAPTER 1. INTRODUCTION

MICROALGAE BIOLOGY, VALUE AND ISOLATION

BIOLOGY OF MICROALGAE

Microalgae are prokaryotic or eukaryotic photosynthetic microorganisms that can grow rapidly and live in a wide range of environmental conditions due to their unicellular or simple multicellular structure (Mata et al. 2010; Boruff et al. 2015). They occur in all existing earth ecosystems, not just aquatic but also terrestrial, representing a large variety of living species. Examples of prokaryotic microalgae are Cyanobacteria (Cyanophyceae) and eukaryotic microalgae are, for example, green algae (Chlorophyta) and diatoms (Bacillariophyta). Together, they are considered responsible for at least 60% of primary production on Earth (Chisti 2004).

The existing number of microalgae in the wild is still unknown (Borowitzka 2013) but the existing quotes report that there may be between 200,000 to several million representatives of those organisms (Pulz & Gross 2004; Guiry 2012). According to Tomaselli (2004), these organisms have traditionally been classified according to the variety of pigments, chemical nature of the products and the reserve components. Cytological and morphological aspects have also been considered, such as the occurrence of flagellated cells, structure of the flagella, processes of formation of nucleus and cell division, presence and characterization of the chloroplast (s) involucres and the possible connection between the endoplasmic reticulum and nuclear membrane. In addition to these, molecular biology techniques have also been used (Hu 2004).

Being ubiquitous organisms, they have adapted to extreme habitats over billions of years of evolution (Norton et al. 1996). This vast variability makes microalgae one of the most promising feedstock's for sustainable supply of commodities for both food and non-food products (Draaisma et al. 2013).

MICROALGAE APPLICATIONS AND MARKET

The evolutionary and phylogenetic diversity of microalgae leads to a great diversity in the chemical composition of these organisms, and therefore, this makes them extremely attractive for bioprospecting and potential exploitation as commercial sources of a wide range of biomolecules (Borowitzka 2013). Therefore, microalgae have been widely recognised as a key value chain for the bioeconomy. The global interest in the biotechnology of microalgae has been followed in Europe, where the SET-PLAN (Strategic Energy Technological Plan) recognizes microalgae of strategic importance for biomaterial production (Gouveia et al. 2015). In addition, past and current European R&D projects also evidence the importance of microalgae biotechnology. As a consequence, microalgae biotechnology has gained considerable progress and relevance in recent decades (Harun et al. 2010). An area that has received increased consideration is the use of microalgae as a feedstock for biofuels (Chisti 2007; Mata et al. 2010; Doan et al. 2011). Many algae species have high lipid content and can be grown on non-arable land using alternate water sources such as seawater. This has led to a current interest in using them as a source of renewable energy and biofuels (Borowitzka & Moheimani 2013). It is known that microalgae can provide feedstock for several different types of renewable fuels such as biodiesel, methane, hydrogen, ethanol, among others (Mata et al. 2010). The production of biofuels associated with environmental benefits, such as CO_2 fixation and wastewater treatment seem to be the future of this area (Pulz & Gross 2004). In fact, the cultivation of microalgae using wastewaters combines economic and environmental advantages leading to a sustainable and economically viable production of biofuels (Sydney et al. 2011; Abdelaziz et al. 2014).

The first use of microalgae by humans dates back 2000 years to the Chinese, who used *Nostoc* to survive during famine (Spolaore et al. 2006). Nowadays, microalgae (including the cyanobacteria) are established commercial sources of high-value chemicals such as β-carotene, astaxanthin, fatty acids, phycobilin, pigments and algal extracts with multiple applications in the cosmeceutical, nutraceutical and functional foods markets (Borowitzka 2013). Currently only a few species of microalgae producing high value products (e.g. *Dunaliella salina*, *Spirulina* sp., *Chlorella* sp.) are of major interest for different regional, national and international markets (Spolaore et al. 2006).

The first commercialized microalgae were *Chlorella* and *Spirulina* as "health food" in Japan, Taiwan and Mexico (Tamiya 1957; Durand-Chastel 1980; Soong 1980). This was followed in the 1980s by the commercialisation of β-carotene from *Dunaliella salina* (Ben-Amotz & Avron 1989) and astaxanthin from *Haematococcus pluvialis* in the 1990s (Lorenz & Cysewski 2000). Carotenoid production represents nowadays one of the most successful markets of microalgae, mainly on the claim of their proven antioxidant properties (Campo et al. 2007). Carotenoids are associated with various health benefits, such as prevention of age-related muscular degeneration, cataract, certain cancers, rheumatoid arthritis, muscular dystrophy and cardiovascular problems. All this emphasizes the interest in finding microalgae species with high carotenoid content (Ahmed et al. 2014). In many markets, microalgal carotenoids are in competition with the synthetic forms of these pigments. Although these are much less expensive than the natural ones, microalgal carotenoids have the advantage of being natural in opposition to the synthetic competitors made through chemical synthesis (Spolaore et al. 2006).

The market for carotenoids in 2010 was about US$1.2 billion with the bulk of the carotenoids being produced by chemical synthesis. β-Carotene and astaxanthin from microalgae do however represent a major part of the 'natural' production of these carotenoids (Borowitzka 2013). The future of microalgal biotechnology for carotenoid production seems, therefore, very promising, and innovative processes and products should be expected to develop in the next few years (Campo et al. 2007).

Microalgae are also an important source of polyunsaturated fatty acids (PUFAs) of more than 18 carbons that are not synthesized *de novo* by higher plants neither animals (Spolaore et al. 2006). The Global Organisation for eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) and Frost and Sullivan (2010) estimated that the global market for EPA and DHA omega-3 oils exceeded 85,000 t in 2009 and was estimated to grow to 135,000-190,000 t by 2015. It has been widely demonstrated that microalgae have the ability to produce a wide range of fatty acids with emphasis on the long chain ones. The presence of long chain poly unsaturated fatty acids (LC-PUFAs) such as eicosapentaenoic acid (C20:5), docosahexanoic acid (C22:6) and the C20:5 precursor, stearidonic acid (C18:4) are of interest because of their benefits for human health (Ratledge 2010; Draaisma et al. 2013). Nevertheless, it is still unknown whether the PUFA-rich oil from phototrophic microalgae can compete with the one from other sources like fish (Borowitzka 2013) as at the moment, the cost of producing microalgae biomass and fuels from microalgae is too high to be competitive with fossil fuels (Stephens et al. 2010; Wijffels & Barbosa 2010).

MICROALGAE ISOLATION

The identification of new species or the development of new strains represents a business opportunity and source of income from the possible royalties resulting from its intellectual property (Mata et al. 2010). There are many algae screening programs around the world surveying different algal species for a variety of applications (Abomohra et al. 2013). Cultivation of microalgae requires good and clean stock cultures that should be preceded by species isolation. Isolation of suitable microalgae from the natural environment has been realized by several methods. Isolation by single cell colony (Murakami & Ikenouchi 1997; Sydney et al. 2011) has been performed to obtain microorganisms from nature with high capability in fixing CO_2 and capable of removing nitrogen and phosphorus. Cytometric cell sorting has been previously reported by Reckermann (2000) and Crosbie et al. (2003) where chlorophyll was used as a fluorescent probe to distinguish between different strains of microalgae. Doan et al. (2011) isolated microalgae strains using green auto-fluorescence (GAF) and chlorophyll auto-fluorescence (CAF) for high biomass productivity.

During the past decades extensive collections of microalgae isolates have been created by researchers in different countries. An example is the freshwater microalgae collection of University of Coimbra (Portugal) considered one of the world’s largest, having more than 4,000 strains and 1,000 species (Mata et al. 2010). A bit all over the world, other algae collections of microalgae isolates attest for the interest that algae have risen, for many different production purposes, such as the collection of the Gottingen University (SAG), the University of Texas Algal Culture Collection and the National Institute for Environmental Studies Collection (NIES) (Mata et al. 2010).

MICROALGAE PRODUCTION

CULTURE SYSTEMS

The growing interest in microalgae biotechnological potential has led to their cultivation. There are two main types of microalgae cultivation systems: open ponds and closed photobioreactors (Borowitzka & Moheimani 2013; Chini et al. 2013), with open ponds the most widely used system for commercial large-scale outdoor microalgae cultivation (Borowitzka 2013).

Open-culture systems are normally less expensive to build and operate, more durable than large closed reactors and with a large production capacity when compared with closed systems (Mata et al. 2010). However according to Richmond (2004), ponds use more energy to homogenize nutrients and the water level cannot be kept much lower than 15 cm (or 150 L m^{-2}) for the microalgae to receive enough solar energy to grow. Generally ponds are more susceptive to weather conditions, not allowing control of water temperature, evaporation and lighting. Also, they may produce large quantities of microalgae, but occupy a more extensive land area and are more susceptible to contaminations from other microalgae or bacteria (Mata et al. 2010). Moreover, since atmosphere only contains approximately 400 ppm CO_2, (Blunden & Arndt 2014) it is expected that mass transfer limitation could slow down the microalgae cell growth.

Currently *Dunaliella salina*, *Spirulina* sp., *Chlorella* sp. (above referred as producing high value products) are grown commercially in open ponds in Australia, China, India, Israel, Japan, Thailand, and the USA, although many other species can be grown reliably in these systems (Boruff et al. 2015). Western Australia hosts the largest commercial microalgae cultivation plant in the world, the *D. salina* plant producing the valuable carotenoid b-carotene. The plant is located in Hutt Lagoon, north of Geraldton along the central coast of Western Australia and comprises a total pond area of over 740 ha (Borowitzka 2013).

The open raceway pond reactor has some drawbacks that limit its use to strains that, by virtue of their weed-like behavior (e.g. *Chlorella*) or by their ability to withstand adverse growing conditions (e.g. *Spirulina* (*Arthrospira*) for high pH values and *Dunaliella* for high salinity), can outcompete other microorganisms (Campo et al. 2007).

Photobioreactors (PBRs) are flexible systems that can be optimized according to the biological and physiological characteristics of the algal species being cultivated, allowing the cultivation of algal species that cannot be grown in open ponds (Mata et al. 2010). Depending on their shape or design, PBRs are considered to have several advantages over open ponds: offer better control over culture conditions and growth parameters (pH, temperature, mixing, CO_2 and O_2), prevent evaporation, reduce CO_2 losses, allow to attain higher microalgae densities or cell concentrations, higher volumetric productivities, offer a safer and protected environment, preventing contamination or minimizing invasion by competing microorganisms (Mata et al. 2010). Photobioreactors (PBRs) are mainly either flat or tubular and can adopt a variety of designs and operation modes (Harun el al. 2010). They offer higher productivity and better quality of the generated biomass (or product), although they are certainly more expensive to build and operate than the open systems (Campo et al. 2007). In a PBR, direct exchange of gases and contaminants (e.g. microorganisms, dust) between the cultivated cells and atmosphere are limited or not allowed by the reactor's walls.

Generally tubular photobioreactors are suitable for outdoor cultures as they are relatively cheap, have a large illumination surface area and have fairly good biomass productivities (Ugwu et al. 2008). Vertical bubble columns and airlift cylinders can attain substantially increased radial movement of fluid that is necessary for improved light–dark cycling. These reactor designs have a low surface/volume, but substantially greater gas hold-ups than horizontal reactors and a much more chaotic gas–liquid flow (Richmond 2004; Ugwu et al. 2008). Other prospects include high mass transfer, good mixing with relatively low shear stress, low energy consumption, high potential for scalability, easy to sterilize, readily tempered, good immobilization of algae. Consequently, cultures suffer less from photoinhibition and photo-oxidation, and experience a more adequate light–dark cycle (Mata et al. 2010).

Calculating the productivity of microalgae is much easier in open ponds than in closed photobioreactors due to their geometry (Moheimani 2013). The productivity of microalgae in open pond culture is usually measured in grams per square meter per day (g m^{-2} day^{-1}) and the best annual average long term productivity that has been achieved to date is approximately 20 g m^{-2} day^{-1} (Borowitzka & Moheimani 2013). Depending on the cultivation system, microalgae concentration is normally between 0.1 and 0.3 g L^{-1} in open ponds (Fon et al. 2011) and 1 and 4 g L^{-1} in some closed photobioreactors (Chisti 2007).

PARAMETERS REGULATING MICROALGAL GROWTH AND REPRODUCTION

For cost-effective cultivation of microalgae, a suitable climate with high annual solar irradiation and an optimum temperature range, which allows year-round microalgae growth at high productivity levels, is critically important (Jonker & Faaij 2013). In general, identification of an appropriate location for microalgae cultivation is a function of microalgae productivity, the physical suitability of the location, the political availability of land, and the affordability of property (Maxwell et al. 1985).

The most important parameters regulating algal growth are quantity and quality of nutrients, light, temperature, pH, turbulence and salinity. The various parameters may be interdependent and differently tolerated by each species (Barsanti & Gualtieri 2014). The ideal location for microalgae cultivation is ultimately based on the specific requirements of the microalgae strain in question (Boruff et al. 2015).

Microalgae biomass productivity is highly dependent upon climate conditions (especially irradiance and temperature range), CO_2 availability, nutrients such as nitrogen and phosphorous, water, and a host of social, economic and political aspects (Fon et al. 2013). Most microalgae mass cultures are carbon limited. The addition of CO_2 can significantly increase biomass (Moheimani 2013). Therefore, CO_2 is a critical component of the cultivation process, the use of which can be facilitated through co-location with a feasible source such as fossil fuel-fired power plants, ammonia production facilities, and cement or waste water treatment plants.

Given that microalgae cultivation is carried out in liquid medium, water is a primary factor of the process. Water consumption has been postulated to be a resource barrier for large-scale production of microalgae (Batan et al. 2013). The amount of water needed for the production of 1 kg of microalgae biomass in closed systems can be estimated from final biomass concentration values. Using the final concentrations given by Yang et al. (2012) of 0.5 g L^{-1} to 7.56 g L^{-1}, and assuming no water loss, the production of 1 kg of biomass consumes between 2,000 and 132 L of water, plus the water needed for the other operations of the process. Borowitzka & Moheimani (2013) reported that due to evaporation rates in regions of high solar irradiation, it is highly unlikely that open pond culture of freshwater microalgae can be used to produce biofuels and the focus for this purpose must be on species able to grow in saline water, preferably over an extended salinity range.

By using seawater or wastewater, the freshwater usage can be reduced by as much as 90% (Yang et al. 2011). However, a significant amount of freshwater must still be used for culture no matter whether sea/wastewater serves as the culture medium or how much harvested water is recycled (Yang et al. 2011). Nevertheless, if algae are grown in an enclosed system such as a photobioreactor, the use of freshwater is feasible as the water lost by evaporation can be recovered and recycled (Borowitzka & Moheimani 2013).

Light is the driving force of photosynthesis, as well as a major issue in cell photo-acclimatization. Both photoperiod and light intensity have a significant effect on microalgal growth. Khoeyi et al. (2012) showed that at different light intensities, an increase in light duration was associated with increased specific growth rates in *C. vulgaris*. Of the various intensities and photoperiods tested, the maximum biomass concentration (2.05 ± 0.1 g L^{-1}) was recorded at a photosynthetic photon flux density (PPFD) of 62.5 µmol photons m^{-2} s^{-1} and 16:8 h. A study of *Tetraselmis chui* by Meseck et al. (2005) suggested that in photoperiods less than 12 h, cell quota, nutrient-uptake and division rate is energy limited, regardless of light intensity.

Experimental investigations reveal that an increase in light intensity is directly proportional to an increase in concentration of cultivated microalgae (Al-Qasmi et al. 2012). However, above a certain threshold, light intensity becomes an inhibiting factor. This photoinhibition phenomenon will generally cause reversible damage to the photosynthetic process (Rubio et al. 2003). Since outdoor microalgal cultures are exposed to environmental conditions, limiting and even inhibiting values for photosynthesis are likely to be recorded on clear days (Carvalho et al. 2011). According to Vonshak et al. (1989), e.g., *Spirulina platensis* becomes “light saturated” well below one quarter of full sunlight and maximum growth rate measured was only at about 20 µmol photons m^{-2} s^{-1}.

Defining the optimal light intensity for the growth of microalgae is complex since it depends on several factors, e.g. species, cultivation system and culture density. Khoeyi et al. (2012) recorded the maximum biomass at a photosynthetic photon flux density (PPFD) of 62.5 µmol photons m^{-2} s^{-1} for a 16:8 h light/dark photoperiod duration in *C. vulgaris*. Kitaya et al. (2005) reported the highest multiplication rate of the microalga *Euglena gracilis* at a PPFD of about 100 µmol m^{-2} s^{-1} with continuous lighting. Chen et al. (2011) observed a reduction in productivity with PPFD above 260 µmol photons m^{-2} s^{-1} in the microalga *Chlorella* sp. The highest biomass production in *Scenedesmus obliquus* was obtained at a photosynthetic photon flux density (PPFD) of 165 µmol photons m^{-2} s^{-1} (Mata et al. 2012).

Temperature is a crucial factor for outdoor production of microalgae. The effect of high temperatures is often described as more deleterious than low temperatures (Ras et al. 2013). Although the optimum temperature is species and strains-specific, most microalgae are capable of carrying out photosynthesis and cellular division over a range of temperatures between 15 and 30°C, with optimal conditions between 20 and 25°C (Li 1980). Kim et al. (2012) demonstrated that optimum temperature conditions for the growth of *Chlorella* and *Dunaliella* strains were 25 and 27°C, respectively. According to Converti et al. (2009), *C. vulgaris* growth appeared to be negatively affected at temperatures above 30°C. At 35°C this microalga exhibited in fact a 17% decrease in its growth rate when compared to 30°C. Further increase in temperature (38°C) led to an abrupt interruption of microalgal growth, and eventually to cell death.

Various culture media have been developed and used for isolation and cultivation of freshwater algae and are useful for growing a wide variety of algae (e.g. Guillard 1975; Becker 1994). The growth medium for microalgal production must provide the inorganic elements that constitute the algal cell. Main elements include nitrogen, phosphorus, iron and in some cases silicon. Nutrients such as phosphorus must be supplied in significant excess because of their reduced bioavailability (Chisti 2007). In commercial cultivation of microalgae, the culture medium represents a significant cost (Mata et al. 2010). In order to reduce production costs and increase commercial viability, many studies have proposed the use of costless nutrients with environmental advantages. Nutrient availability in the form of nitrogen and phosphorus can be supplied as traditional fertilizers or potentially from sources such as food production facilities, waste water treatment plants or as agricultural runoff (Boruff et al. 2015).

MICROALGAE PRODUCTION IN THE AZORES

The Azores archipelago, relatively isolated in the mid-Atlantic Ocean (36° 55’ to 39° 43’ N and 24° 45’ to 31° 17’ W), is known by its many freshwater bodies. The Azorean freshwater algal flora has been described as species poor, dominated by species with cosmopolitan distribution (Bohlin 1901; Bourrelly & Manguin 1946a, 1946b). However, taxonomic studies on Azorean freshwater algae are rare and, to our knowledge, those organisms have not before been isolated neither subject of biotechnological investigations. Due to the isolation and remoteness of the archipelago, a high degree of endemism could be expected as it has been observed on other remote islands, e.g. Falkland (Flower 2005), Hawaii (Sherwood et al. 2014), maritime Antarctic region (Vijver & Beyens 1997).

The ideal microalgal characteristics are that it must grow well even under high cell density and under varying outdoor environmental conditions and be able to have high biomass productivity (Moheimani 2013). Native species are supposed to be adapted to local environmental changes and thus resilient and competitive. These are critical characteristics supporting the success of large-scale production (Nascimento et al. 2013). Bioprospection of autochthonous algal species may be considered as an emergent research activity in many countries (Mutanda et al. 2011). Recently the term phycoprospecting was introduced to explain that native microalgae species have long been naturally selected to their local regions. Local species are a priori adapted to the prevailing abiotic and biotic factors, and thus are evolutionarily primed for local bioresource productions (Wilkie et al. 2011). Furthermore, the choice of local species minimizes any environmental impact in the event of accidental release from large-scale commercial culture (Mohsenpour et al. 2012).

The Azores have the human capital and the political will to take advantage of opportunities in the field of microalgae production. The University of the Azores (UAc) is a higher education institution with centres on three islands (Faial, Terceira and São Miguel). The education offer covers a range of courses related to biotechnology such as biology, engineering, or pharmaceutical sciences. Research areas of the UAc include biochemistry, aquaculture and algal biology. A pilot project of microalgae cultivation in partnership with a start-up company (ALGICEL) gave positive results which are now being consolidated. The Azores Government promotes a number of investment incentives that aim to promote the sustainable development of the regional economy, including research-based innovation. In fact, biotechnology and aquaculture are among the strategic priorities set out in the research and innovation strategy for smart specialisation of the region (SPI AÇORES 2014).

Thesis aims and outline

Given the global and regional scenarios, in which there is increasing interest in the biotechnology of microalgae, this work has the following general objectives:

- to analyse the international and regional context for outdoor microalgae production and evaluate the potential of the Azores in this area;
- the survey, isolation and establishment of culture collections of indigenous Azorean microalgae to build the basis for subsequent taxonomic and biotechnological studies;
- to evaluate the outdoor culture potential of indigenous microalgae species in terms of biomass productivity, fatty acids and carotenoids;
- to optimize the growth and secondary carotenoid production of selected indigenous microalgae in outdoor cultures using vertical tubular photobioreactors.

The evaluation of the feasibility of local outdoor microalgae production in the Azores is undertaken in Chapter 2 that describes the international and regional context for outdoor microalgae production and uses a SWOT analysis to analyse the potential of this region in this area. Some of the local natural conditions favour the development of commercial microalgae production, given that they surpass many of the basic requirements of this activity. Apart from the natural conditions, some of the activities already present in the region are also identified as strengths.

Knowing that there are advantages in using native species for the production of microalgae, Chapter 3 describes the survey, isolation and establishment of a culture collection of indigenous microalgae. 114 isolates of microalgae were obtained from samples collected in 23 locations. From them, 60 taxa were identified comprising 39 Chlorophyta, 10 Ochrophyta, 6 Cyanophyta, 3 Charophyta, 1 Euglenozoa and 1 Cryptophyta. Eighteen of the taxa identified constitute new records for the Island of São Miguel. This work is the first of its kind in the Region and served to build the basis for subsequent taxonomic and biotechnological studies.

The exploration of indigenous microalgae species with biotechnological potential is growing due to advantages in their use. In Chapter 4 the outdoor culture potential of five indigenous microalgae species is evaluated and the composition of the biomass produced in terms of fatty acids and secondary carotenoids analysed. *Chlorococcum* sp. was found to be the most interesting strain due to its significantly higher production of both unsaturated fatty acids and secondary carotenoids. The values obtained compared favourably with those of commercially used strains.

Given the results obtained in the previous chapter, Chapter 5 addresses the effect of nutrients and bicarbonate concentration on the growth and carotenoid accumulation on an indigenous strain of *Chlorococcum* sp. The study demonstrated that this strain has potential for a commercial outdoor production of secondary carotenoids using bicarbonate as a carbon source.

Chapter 6 summarizes the main conclusions of the research performed and suggests future research in the area.

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