PREFACE

Microalgae are microscopic algae, typically found in freshwater and marine systems. Microalgae, capable of performing photosynthesis, are important for life on earth; they produce approximately half of the atmospheric oxygen and use simultaneously the greenhouse gas carbon dioxide to grow photoautotrophically. The biodiversity of microalgae is enormous and they represent an almost untapped resource. In this book, the authors present current research in the study of microalgae, including microalgal biotechnological applications in nutrition, health and the environment; using microalgae biomass for biodiesel and biofuel production and microalgae for aquaculture.

Chapter 1 - Microalgae (prokaryotic and eukaryotic) consist of a wide range of autotrophic organisms which grow through photosynthesis just like land based plants. Their unicellular structure allows them to easily convert solar energy into chemical energy through CO_2 fixation and O_2 evolution, being well adapted to capture CO_2 and store it as biomass. Microalgae and cyanobacteria have an interesting and not yet fully exploited potential in biotechnology. They can be used to enhance the nutritional value of food and animal feed due to their chemical composition, playing a crucial role in aquaculture. Highly valuable molecules like natural dyes (e.g. carotenoids), polyunsaturated fatty acids, polysaccharides and vitamins from algal origin are being exploited and can be applied in the nutritional supplements; cosmetics (e.g. phycocyanin) and pharmaceuticals. In fact, microalgae and cyanobacteria are able to produce several biologically active compounds with reported antifungal, antibacterial, anticancer, antiviral (e.g. anti-HIV), immunosuppressive, anti-inflamatory and antioxidant activity. Nowadays, there is a focus on using microalgae in renewable energy sources and environmental applications. Microalgae are a potential source for biofuels production such as biodiesel, bioetanol, biohydrogen and biogas. These can be produced through a biorefinery concept, in which every component of the biomass is used to produce usable products. This strategy can integrate several different conversion technologies (chemical, biochemical, termochemical and direct combustion) providing a higher cost effective and environmental sustainability for the biofuels production. Environmental applications can include CO_2 sequestration and wastewater treatment. This can be achieved by coupling microalgae production systems with industrial polluting facilities.

Chapter 2 - Using process modelling tools, the conventional and in-situ transesterification processes for biodiesel production from microalgae biomass was modelled.The raw material and process energy requirements of the up-scaled process were obtained for the different transesterification processes, and a renewability assessment of the various schemes was carried out.The biomass cultivation and biodiesel production process renewability was assessed by comparing the minimum work required to restore the non-renewable resources degraded in the biomass and biodiesel production process with the useful work available from the main process products. If the maximum work obtained from the process products is larger than the restoration work, the process is considered as renewable. In a present day scenario (with the use of fossil fuel sources for the production of the process raw materials, such as for methanol and sulphuric acid production, and electricity), all the transesterification processes were shown to be non-renewable. The influence of the choice of the electricity generation scheme, raw material source and the type of heating fuels (including heating and drying technology) on the process renewability was also examined. The process renewability of the in-situ transesterification of microalgae lipids to biodiesel was found to significantly improve with the use of renewable electricity, reacting alcohols from biomass fermentation and heat pump technology to facilitate the biomass drying and process heating.

Chapter 3 - The ubiquity and persistence of organic pollutants in the aquatic environment are of potential risk to aquatic habitats and human health due to their highly toxic, mutagenic and carcinogenic properties. Bioremediation, a cost-effective technology to remove organic pollutants from contaminated water bodies, involves a number of biological processes, including accumulation, transformation and degradation, mediated mainly by microorganisms such as bacteria, fungi and microalgae. Eukaryotic microalgae are dominant primary producers and play a central role in the fixation and turnover of carbon and other nutrient elements. However, their role in the remediation of aquatic contaminants and the relative importance of the processes involved are much less understood, as compared to bacteria and fungi. Further, most of the studies on the novel remediation technology using microalgae have concentrated on metals and nutrients and much less on organic pollutants. Screening of tolerant species is a crucial step in bioremediation, and the understanding of the response and adaptive changes in microalgal cells to toxicity induced by organic pollutants is equally important as it serves as a scientific basis of remediation practices. However, there has been very little published information on the toxicity, resistance and adaptations of microalgae to persistent organic pollutants (POPs). This paper reviews the recent research on the sensitivity, tolerance and adaptations of microalgae to the toxicity of various POPs, including organochlorine pesticides (OCPs), polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs), polybrominated diphenyl ethers (PBDEs) and some other emerging environmental endocrine disrupting compounds. The review focuses on the physiological and biochemical changes in microalgae and their relationships with tolerance. The mechanisms and factors affecting the capacity of microalgal cells to remove POPs, as well as the feasibility, limitations and future research directions of employing microalgae in POPs remediation, are also addressed.

Chapter 4 - Many analyses have been carried out about the future possibility of exhausting the planet's resources and its ability to sustain its inhabitants. The use of microorganisms and their metabolic products by humans is one of the most significant fields of biotechnology activities. Microalgae are descendants of the first photosynthetic life forms. More than 3,500 million years ago the oxygen atmosphere was made up by cyanobacteria and other forms of life could evolve. Since then, microalgae have contributed to regulating the planet's biosphere. The use of solar energy through photosynthesis in microalgae cultivation is a clean, efficient and low cost process, because the sun's energy is virtually free and unlimited. The biomass of microalgae and its products are employed in many fields: feed and food additives in agriculture, fertilizers, in the food industry, pharmaceutics, perfume making, medicine, in biosurfactants, biofuels and in science. This wide use is due to its fast growth, non-toxicity, assimilability (85–95%), high protein content (60–70%), well-balanced amino acid composition, richness in vitamins, minerals, fatty acids, biopigments, biopolymers, and the fact that is has a great variety of biologically active agents in appreciable amounts. Microalgae are considered to be efficient immunopotentiators and have anticarcinogenic and antiviral effects. Producing biocompounds from microalgae has the additional advantage of simultaneously fixing large amounts of carbon dioxide. The use of these alternative sources reduces costs and can generate carbon credits. Microalgae can be grown on land that is unsuitable for agriculture and farming, or on inhospitable land such as deserts, using brackish water and/or wastes from the desalination process. The composition and rates of photosynthesis and growth of these organisms are strongly dependent on growth conditions. Manipulating these conditions can result in higher yield metabolites that are of interest. At the end of the process, according to the characteristics of the microalgal biomass obtained, it can be used to produce different compounds. The objective of this study was to present traditional and advanced bioproducts obtained from microalgal biomass and to describe the characteristics of the cultivation process.

Chapter 5 - Hydrothermal carbonization is a process in which biomass is heated in water under pressure at temperatures below 250 °C to create a char product. A significant advantage of the process is that water is not removed from the char by evaporation but by filtration, providing a favorable energy input to output ratio.

With higher plants, the chemistry derives primarily from lignin, hemicellulose and cellulose components. Cellulose is the most recalcitrant of these and requires relatively high temperatures and long reaction periods for conversion into a highly carbonized char product.

The author's approach has been to examine biomass materials having relatively low cellulose contents, applying reaction temperatures generally below 225 °C, and for reaction periods of less than 2 hours. These conditions are believed to be conducive to continuous processing and to increase the carbon content in the char primarily via a dehydration mechanism, rather than by loss of carbon dioxide. Substrates that have been examined in this manner included microalgae, cyanobacteria, and fermentation residues such as distiller's grains, brewer's grains and others.

It was determined during the course of these investigations that fatty acids created by hydrolysis of lipids during the process do not chemically contribute to char formation but are adsorbed onto the char and can be recovered by solvent extraction in high yield. Therefore, the process provides fatty acid, char, and aqueous filtrate products, all of which have utility.

Chapter 6 - Despite of its high cost, the use of live feed is essential and frequently irreplaceable in the aquaculture of mollusks, fishes, crustaceans, and some other aquatic organisms, especially in the larviculture and nursery phases (Lin et al. 2009). The use of microalgae during these phases seems to be a universal practice, because some microalgae species have adequate physical and nutritional characteristics for the early development of aquatic organisms, and the operative costs for their production is commonly lower compared to the production of other organisms or formulated feeds (Martínez Córdova et al. 1999; Lovatelli et al. 2004).

Chapter 7 - Carbon neutral renewable source of energy is needed to displace petroleum-derived fuels, which contribute to global warming and are of limited availability. Biodiesel and bioethanol, in this context, are the two potential renewable fuels that have gained substantial attraction. However, sustainability of biodiesel and bioethanol production from conventional agricultural crops is still questionable. Microalgae, a source of biodiesel are at the center of new research conducted with the aim of completely displacing fossil-based diesel. With special reference to biodiesel, the present article reviews prospects and constraints of microalgae as a source of biofuel.

There are at least 30,000 known species of microalgae, of which only a handful are currently of commercial significance due to their non-energy products such as nutraceuticals, pigments, proteins and functional foods. Though may vary with the species, microalgal biomass can be rich in proteins or rich in lipids or have a balanced composition of lipids, sugars and proteins. Under laboratory conditions, some microalgae strains were reported to generate 70 % lipid in their biomass. The fundamental chemical reaction required to produce biodiesel is the esterification of lipids, either triglycerides or oil, with alcohol, which results in a fatty acid alkylester called biodiesel (Fatty acid methyl-ester). As the fastest growing photosynthesizing organisms, biomass harvest of microalgae (158 tons/ha) is significantly higher than that of crop species such as sugarcane (75 tons/ha) used for bioethanol production. Under optimum growing conditions, a hectare of microalgae may potentially yield about 8,000 liters of biodiesel, which is 10 to 1000 times as much liquid fuel per year per hectare as conventional crops.

However, achieving the capacity to inexpensively produce biodiesel from microalgae is still challengeable. It could therefore be concluded that though microalgae are considered to be a potential source of green energy, the sustainability will largely depend on development of cost effective culture and processing techniques. Screening and collecting strains of algal species to access their potential for high oil production with high biomass productivity, investigating the physiology and biochemistry of the algae, use of molecular-biology and genetic engineering techniques to enhance the oil yield and development of advance processing techniques of cost competitive are considered to be the priority areas of research concern.

Chapter 8 - Single-cell algae (microalgae) are among the most promising resources for the production of biofuels and bioactive compounds, as well as for CO_2biomitigation and bioremediation. Improvement of microalgal photobiotechnologies for the production of value-added products such as long-chain polyunsaturated fatty acids, storage triacylglycerols and carotenoids, requires fast and reliable, and preferably non-destructive techniques for on-line monitoring of the target product's content and the physiological condition of the algal culture. These techniques can provide essential information for timely and informed decisions on adjusting illumination conditions and medium composition, and on the optimal time for biomass harvesting. Often, such decisions must be taken within hours, and mistakes can lead to a significant reduction in productivity or a total loss of the culture. A promising approach for real-time non-destructive monitoring of laboratory and upscaled microalgal cultures is based on measuring the optical properties of algal suspensions, such as absorption, scattering and reflection of light by microalgal cells in certain spectral regions. To this aim, the following criteria should be met: i) reliable spectral measurements, ii) efficient algorithms for the processing of spectral data, and iii) a thorough understanding of the relationships between changes in physiological condition and/or biochemical composition of the algal culture and accompanying changes in its optical properties. This chapter presents a review of recent experimental work in this area, with an emphasis on investigations conducted by the authors and their colleagues in the fields of physiology, biochemistry and spectroscopy which have implications for the cultivation of biotechnologically important microalgal species.

Chapter 9 – The worldwide fossil fuel reserves are on the decline but the fuel demand is increasing remarkably. The combustion of fossil fuels needs to be reduced due to several environmental concerns. Biofuels are receiving attention as alternative renewable and sustainable fuels to ease our reliance on fossil fuels. Biodiesel and bioethanol, the two most successful biofuels in the transport sector, are currently produced in increasing amounts from oil or food crops, but their production on a large scale competes with world food supply and security. Microalgae offer a favourable alternative source of biomass for biofuel production without compromising land and water resources since they can be easily cultured on waste land which cannot support agriculture. This chapter focuses on the potential of microalgal biomass for production of the transport fuels, biodiesel and bioethanol and the bottlenecks and prospects in algal fuel technology.

Chapter 10 - While human beings are combating against global warming, fuel shortage, resource depletion, and economic downturn, microalgae, the oldest plants on earth, are gaining intensive and unprecedented attentions. The broad variety, wide distribution, and versatile growth conditions allow microalgae to be used in various fields. To be more specific, microalgae can assist humans in solving many of the challenges we are facing. But taking advantages of their unique capabilities requires better knowledge of them. Different microalgae thrive in different environment. This review focuses on identifying the best species/strains for sequestering CO_2 from flue gas released from stationary sources. Though no complete studies have been conducted for selected strains, this review helps to narrow the range and pave the way for future in-depth investigations of well-suited microalgal species in terms of capturing CO_2 and developing value-added products.

Chapter 11 - An important amount of the applied load of pesticides enter into aquatic ecosystems from agricultural runoff or leaching and, as a consequence, have become some of the organic pollutants that appear most frequently on aquatic ecosystems. The assessment of toxic potential in surface water is one of the main tasks of environmental monitoring for the control of pollution. Animal organisms such as fishes or mussels have been examined intensively whereas little information is available on the susceptibility of water plants and plankton organisms.

As primary producers, microalgae constitute the first level of aquatic trophic chains. Due to its microscopic size, it is possible to get sample at population and community levels. Some species can be cultivated in photobioreactors under controlled conditions. Because of their short generation times, microalgae respond rapidly to environmental changes, and any effect on them will affect to higher trophic levels. In addition, microalgae offer the possibility to study the trans-generational effects of pollutant exposure, being a model of choice for the study of the long term effects of pollutant exposure at population level. Furthermore, microalgal tests are generally sensitive, rapid and low-cost effective. For all these reasons, the use of microalgal toxicity tests is increasing, and today these tests are frequently required by authorities for notifications of chemicals and are also increasingly being used to manage chemical discharges. For example, algal toxicity tests of chemicals are mandatory tests for notification of chemicals in the European Union countries. Others fields of use for algae in toxicity assessment are industrial wastewaters and leachates from waste deposits.

Cytotoxic effects of aquatic pollutants on microalgae are very heterogeneous, and they are influenced by the environmental conditions and the test species. Growth, photosynthesis, chlorophyll fluorescence and others parameters reflect the toxic effects of pollutants on microalgae; however, other relevant endpoints are less known because experimental difficulties, especially under in vivo conditions.

During the last two decades, our research group has a high priority scientific objective: study the effect of different aquatic pollutants on freshwater microalgae, with the aim to develop new methods for the detection of contaminants based on the physiological response of microalgae, with the purpose of providing an early warning signal of sublethal levels of pollution.

Chapter 12 - This study investigated co-solvent modified supercritical carbon dioxide (SC-CO_2) extraction of lipids and carotenoids from the microalgal species of *Nannochloropsis oculata*. The changes in content of zeaxanthin in submicronized precipitates generated from the supercritical anti-solvent (SAS) process were also examined. The effect of operational conditions on amount, recovery of the zeaxanthin and mean size, morphology of the precipitates was obtained from experimentally designed SAS process. The mean size of particles falls within several hundreds of nano meters and is highly dependent on the injection time, the content of zeaxanthin in the particulates ranged from 65 to 71%. Finally, the biological assays including antioxidant and anti-tyrosinase abilities were tested to evidence the bioactivity of zeaxanthin. This study demonstrates that elution chromatography coupled with a SAS process is an environmentally benign method to recover zeaxanthin from *N. oculata* as well as to produce nanosize particles containing zeaxanthin from algal solutions.

Chapter 13 - Biological colonisation of stone is one of the main problems related to monuments and buildings conservation. It is amply recognised that microalgae have the greatest ecological importance as pioneer colonisers of stone materials, conducting to aesthetic, physical and chemical damages. Their deterioration potential is related with their photoautotrophic nature, using the mineral components of stone substrates and sunlight as energy source without any presence of organic matter.

Stone biodeterioration by microalgae has been assessed by several authors. Most of the employed methodologies for microbial identification and monitoring are time-consuming and require extensive sampling. In addition, the scaffolding and sampling procedures required may also transform the researcher in a biodeteriorating agent itself. In this chapter, non-contact techniques for colonisation detection and monitoring are proposed in order to fulfil the mission of heritage preservation. *In vivo* chlorophyll *a* fluorescence and digital image analysis were applied to estimate microalgal biomass and to quantify coverage of limestone samples artificially colonised by algal communities. The results showed that *Ançã* and especially *Lecce* limestones were extensively colonised on their surfaces revealing significant epilithic growth, whereas *Escúzar* and *San Cristobal* limestones were endolithically colonised by photoautotrophic microorganisms.

The easily handled, portable and non-destructive techniques proposed allow the understanding of stone biodeterioration processes avoiding contact and damaging of the objects, which ensures a wide field of application on cultural heritage studies and the design of appropriate conservation and maintenance strategies.

Chapter 14 - Carotenoids are isoprenoid polyene pigments widely distributed in nature. They are the main source of the red, orange or yellow colour of many edible fruits (lemons, peaches, apricots, oranges, strawberries, cherries, and others), vegetables (carrots and tomatoes), mushrooms (milk-caps), and flowers. They are also found in animal products:

eggs, crustaceans (lobsters, crabs and shrimps) and fish (salmonids) (De Saint Blanquat, 1988).

Chapter 15 - Green microalgae biomass would represent in tropical countries an innovative proteinaceous bioresource for developing protein hydrolysates. The proteolytic modification could have special importance for the improvement of solubility of algal protein and for decreasing its residual antigenicity. This chapter examined the nitrogen solubility, residual antigenicity and safety of *Chlorella vulgaris* protein hydrolysate (Cv-PH). A high increase of nitrogen solubility in Cv-PH, with respect to *Chlorella* aqueous extract (Cv-EA) was observed over a wide pH range (2-8). Residual antigenicity of Cv-PH was measured using male guinea pigs sensitized with Cv-EA. Neither mortality nor positive anaphylaxis symptoms were observed in Cv-PH challenged animals. The safety of Cv-PH was evaluated in an oral acute toxicity study (OECD Guideline 423) and in a 28-day repeated dose oral toxicity study (OECD Guideline 407) using mice as an experimental animal model. In the acute toxicity study (at a dose of 2 000 mg/kg) neither mortality nor changes in general condition were observed over a 14-days observation period. In the repeated dose oral toxicity study (a limit test at a dose of 2 000 mg/kg) no clinical changes were found in the experimental animals. The increased hemoglobin levels and leukocyte counts, particularly neutrophils, observed in Cv-PH groups may be related to the hemopoiesis stimulatory effect reported previously in *Chlorella* protein hydrolysates. Organ weights at the end of experimentation and histopathological tests revealed no significant influence of Cv-PH. These findings indicate the safety of Cv-PH in preclinical studies. Since there were no observed adverse effects of Cv-PH in these studies, the NOAEL (no observed adverse effect level) for *Chlorella* protein hydrolysate is 2 000 mg/kg/day administered orally for 28 days. An extended knowledge of the functional properties and safety of microalgae hydrolysates can be useful in understanding their potential use in the food and pharmaceutical industries.

Chapter 16 - Heterotrophic microalgal species can be grown in processes and in bioreactors resembling what is used to grow the more common types of industrial microorganisms, bacteria, yeast, and fungi. This opportunity gives heterotrophic microalgal cultures some advantages over phototrophic microalgal cultures in terms of productivity and hygienic standard. Phototrophic microalgal and cyanobacterial cultures are typically 1-2 orders of magnitude less productive than what is often obtained in heterotrophic cultures, partly because only limited amounts of light can be supplied to these cultures, and partly because inhomogeneous light intensities inside the cultures result in low photosynthetic yields near culture surfaces and no photosynthetic activity in central zones too deep to be reached by light. It is also less problematic to maintain cultures axenic in ordinary bioreactors with more compact designs than large-scale photobioreactors, where large surface areas are needed to maximise the collection of light. Heterotrophic cultures are not influenced by climate and weather, in contrast to sunlight dependent, large-scale phototrophic cultures located outdoors.

Cultivation of heterotrophic microalgae is, however, also not unproblematic. Heterotrophic microalgae grow more slowly than many bacteria and yeasts, and heterotrophic microalgae are therefore mainly of interest if they produce something that is not made by other types of microorganisms. Only a limited number of microalgal species will grow heterotrophically, and the number of heterotrophic microalgae synthesising valuable products that cannot be obtained also from other sources is low. Still, heterotrophic species from a phylogenetically highly diverse selection chlorophytes, rhodophytes, cyanidiophytes, diatoms, heterokontophytes, euglenoids, and dinoflagellates have been or are being developed for productions of food, feed, lipids, pigments, and more. A few heterotrophic microalgal processes have also matured to commercialisation. Green algae of the genus *Chlorella* are produced heterotrophically and used as health food, and docosahexaenoic acid, an essential ω-3 poly-unsaturated fatty acid is produced in the dinoflagellate *Crypthecodinium cohnii* and the thraustochytrids *Schizochytrium* sp. and *Ulkenia* sp. and added to infant formula and foods.

Chapter 17 - The increase of atmospheric carbon dioxide is considered to be one of the main causes of global warming. Between 1990 and 2008, atmospheric CO_2 rose from 280 ppm (20.541 mill. t) to 400 ppm (29.381 mill. t). At the same time, fossil oil resources are said to be depleted within a few decades if fuel consumption remains at current levels. It is, therefore, crucial to explore alternatives to oil producing sources and also to reduce atmospheric CO_2 concentration.

Microalgae have been suggested as excellent candidates meeting the requirements: they are able to fix large amounts of carbon dioxide and transform it into biomass with high content of lipids.

The lipid content of microalgae is characteristic of their genus and species and also depends on the different growth phases and culture conditions like nutrient supply (especially nitrogen and phosphate amounts in culture medium), light intensity, temperature, pH and carbon dioxide concentration.

Different microalgae species of the division of Chlorophyta (*Scenedesmus* sp. and *Chlorella* sp.) were investigated according to their biomass productivity, lipid content, fatty acid profile and tolerance of high levels of carbon dioxide. The study showed that a higher CO_2 level leads to a decrease in biomass concentration and an increase in lipid content of the analysed species. For the most part, lipids contain saturated and unsaturated fatty acids with a chain length between C14 and C22. With increasing CO_2 concentration, the content of unsaturated fatty acids with 1 and 2 double bonds increases, whereas the content of linolenic acid, an acid with 3 double bonds, decreases.