PREFACE

Cyanobacteria, also known as blue-green algae, blue-green bacteria or cyanophyta, is a phylum of bacteria that obtain their energy through photosynthesis. They are a significant component of the marine nitrogen cycle and an important primary producer in many areas of the ocean, but are also found in habitats other than the marine environment; in particular, cyanobacteria are known to occur in both freshwater and hypersaline inland lakes. They are found in almost every conceivable environment, from oceans to fresh water to bare rock to soil. Cyanobacteria are the only group of organisms that are able to reduce nitrogen and carbon in aerobic conditions, a fact that may be responsible for their evolutionary and ecological success. Certain cyanobacteria also produce cyanotoxins. This new book presents a broad variety of international research on this important organism.

Chapter 1 - Photosystem I (PS I) is large pigment-binding multi-subunit protein complex essential for the operation of oxygenic photosynthesis. PS I is composed of two functional moieties: a functional core which is well conserved throughout evolution and an external light harvesting antenna, which shows great variability between different organisms and generally depends on the spectral composition of light in specific ecological niches. The core of PS I binds all the cofactors active in electron transfer reaction as well as about 80 Chlorophyll a and 30 β-carotene molecules. However, PS I cores are organised as a supra-molecular trimer in cyanobacteria differently from the monomeric structure observed in higher plants. The most diffuse outer antenna structures are the phycobilisomes, found in red algae and cyanobacteria and the Light Harvesting Complex I (LHC I) family found in green algae and higher plants. Crystallographic models for PS I core trimer of *Synechococcus elongatus* and the PS I-LHC I super-complex from pea have been obtained with sufficient resolution to resolve all the cofactors involved in redox and light harvesting reaction as well as their location within the protein subunits framework. This has opened the possibility of refined functional analysis based on site-specific molecular genetics manipulations, leading to the discovery of unique properties in terms of electron transfer and energy transfer reaction in PS I. It has been recently demonstrated that the electron transfer cofactors bound to the two protein subunits constituting the reaction centre are active in electron transfer reactions, while only one of the possible electron transfer branch is active in Photosystem II and its bacterial homologous. Moreover, Photosystem I binds chlorophyll antenna pigments which absorb at wavelength longer than the photochemical active pigments, which are known as red forms. In cyanobacteria the red forms are bound to PS I core while in higher plants are located in the external LHC I antenna complexes. Even though the presence of the long-wavelength

chlorophyll forms expands the absorption cross section of PS I, the energy of these pigments lays well below that of the reaction centre pigments and might therefore influence the photochemical energy trapping efficiency. The detailed kinetic modelling, based on a discrete number of physically defined compartments, provides insight into the molecular properties of this reaction centre. This problem might be more severe for the case of cyanobacteria since the red forms, when present, are located closer in space to the photochemical reaction centre. In this chapter an attempt is presented to reconcile findings obtained in a host of ultra-fast spectroscopic studies relating to energy migration and electron transfer reactions by taking into account both types of phenomena in the kinetics simulation. The results of calculations performed for cyanobacterial and higher plants models highlights the fine tuning of the antenna properties in order to maintain an elevated (>95%) quantum yield of primary energy conversion.

Chapter 2 - The cyanobacterium *Spirulina* is well recognized as a potential food supplement for humans because of its high levels of protein (65-70% of dry weight), vitamins and minerals. In addition to its high protein level, *Spirulina* cells also contain significant amounts of phycocyanin, an antioxidant that is used as an ingredient in various products developed by cosmetic and pharmaceutical industries. *Spirulina* cells also produce sulfolipids that have been reported to exert inhibitory effects on the *Herpes simplex* type I virus. Moreover, *Spirulina* is able to synthesize polyunsaturated fatty acids such as glycerolipid γ-linolenic acid (GLA; $C18:3^{\Delta 9,12,6}$), which comprise 30% of the total fatty acids or 1-1.5% of the dry weight under optimal growth conditions. GLA, the end product of the desaturation process in *Spirulina*, is a precursor for prostaglandin biosynthesis; prostaglandins are involved in a variety of processes related to human health and disease. *Spirulina* has advantages over other GLA-producing plants, such as evening primrose and borage, in terms of its short generation time and its compatibility with mass cultivation procedures. However, the GLA levels in *Spirulina* cells need to be increased to 3% of the dry weight in order to be cost-effective for industrial scale production. Therefore, extensive studies aimed at enhancing the GLA content of these cyanobacterial cells have been carried out during the past decade.

As part of these extensive studies, molecular biological approaches have been used to study the gene regulation of the desaturation process in *Spirulina* in order to find approaches that would lead to increased GLA production. The desaturation process in *S. platensis* occurs through the catalytic activity of three enzymes, the Δ^9, Δ^{12} and Δ^6 desaturases encoded by the *desC*, *desA* and *desD* genes, respectively. According to authors previous study, the cellular GLA level is increased by approximately 30% at low temperature (22°C) compared with its level in cells grown at the optimal growth temperature (35°C). Thus, the temperature stress response of *Spirulina* has been explored using various techniques, including proteomics. The importance of *Spirulina* has led to the sequencing of its genome, laying the foundation for various additional studies. However, despite the advances in heterologous expression systems, the primary challenge for molecular studies is the lack of a stable transformation system. Details on the aspects mentioned here will be discussed in the chapter highlighted *Spirulina*: Biotechnology, Biochemistry, Molecular Biology and Applications.

Chapter 3 - Cyanobacteria are prokaryotic oxygen-evolving photosynthetic organisms which had developed a sophisticated linear electron transport chain with two photochemical reaction systems, PSI and PSII, as early as a few billion years ago cyanobacteria. By endosymbiosis, oxygen-evolving photosynthetic eukaryotes are evolved and chloroplasts of

the photosynthetic eukaryotes are derived from the ancestral cyanobacteria engulfed by the eukaryotic cells. Cyanobacteria employ phycobiliproteins as major light-harvesting pigment complexes which are brilliantly colored and water-soluble chromophore-containing proteins. Phycobiliproteins assemble to form an ultra-molecular complex known as phycobilisome (PBS). Most of the PBSs from cyanobacteria show hemidiscoidal morphology in electron micrographs. The hemidiscoidal PBSs have two discrete substructural domains: the peripheral rods which are stacks of disk-shaped biliproteins, and the core which is seen in front view as either two or three circular objects which arrange side-by-side or stack to form a triangle. For typical hemidiscoidal PBSs, the rod domain is constructed by six or eight cylindrical rods that radiate outwards from the core domain. The rods are made up of disc-shaped phycobiliproteins, phycoerythrin (PE), phycoerythrocyanin (PEC) and phycocyanin (PC), and corresponding rod linker polypeptides. The core domain is more commonly composed of three cylindrical sub-assemblies. Each core cylinder is made up of four disc-shaped phycobiliprotein trimers, allophycocyanin (AP), allophycocyanin B (AP-B) and AP core-membrane linker complex (AP-L_{CM}). By the core-membrane linkers, PBSs attach on the stromal side surface of thylakoids and are structurally coupled with PSII. PBSs harvest the sun light that chlorophylls poorly absorb and transfer the energy in high efficiency to PSII, PSI or other PBSs by AP-L_{CM} and AP-B, known as the two terminal emitters of PBSs. This directional and high-efficient energy transfer absolutely depends on the intactness of PBS structure. For cyanobacteria, the structure and composition of PBSs are variable in the course of adaptation processes to varying conditions of light intensity and light quality. This feature makes cyanobacteria able to grow vigorously under the sun light environments where the photosynthetic organisms which exclusively employ chlorophyll-protein complexes to harvesting sun light are hard to live. Moreover, under stress conditions of nitrogen limitation and imbalanced photosynthesis, active phycobilisome degradation and phycobiliprotein proteolysis may improve cyanobacterium survival by reducing the absorption of excessive excitation energy and by providing cells with the amino acids required for the establishment of the 'dormant' state. In addition, the unique spectroscopic properties of phycobiliproteins have made them be promising fluorescent probes in practical application.

Chapter 4 - *Prochloron* is an oxygenic photosynthetic prokaryotes that possess not only chlorophyll *a* but also *b* and lacks any phycobilins. This cyanobacterium lives in obligate symbiosis with colonial ascidians inhabiting tropical/subtropical waters and free-living *Prochloron* cells have never been recorded so far. There are about 30 species of host ascidians that are all belong to four genera of the family Didemnidae. Asicidian-cyanobacteria symbiosis has attracted considerable attention as a source of biomedicals: many bioactive compounds were isolated from photosymbiotic ascidians and many of them are supposed to be originated from the photosymbionts. Since the stable *in vitro* culture of *Prochloron* has never been established, there are many unsolved question about the biology of *Prochloron*. Recent genetic, physiological, biochemical, and morphological studies are partly disclosing various aspects of its enigmatic life, e.g., photophysiology, metabolite synthesis, symbiosis, and evolution. Here, authors tried to draw a rough sketch of the life of *Prochloron* and some related cyanobacteria.

Chapter 5 - Cyanotoxin contamination in ichthyic fauna is a worldwide occurrence detected in small aquacultures and natural lakes, underlying a new class of risk factors for consumers. Microcystin contamination in fish tissues is a recent finding in Italian lakes,

Microcystins were analysed by HPLC-PDA using a Dionex Summit system, which includes a high pressure gradient pump Dionex Summit, an autosampler Dionex ASI-100, a column oven Dionex STH-585 and a photo diode-array detector Dionex PDA-100. A C18 column was used (Merck Purospher STAR RP-18 endcapped, 3 μm particles, LiChroCART 55x4 mm). The mobile phase used a gradient of milli-Q water and acetonitrile, both with 0.05% (v/v) of trifluoracetic acid. Chromatograms were analysed between 180 – 900 nm, with a main detection at 238 nm for the typical microcystins spectra (Meriluoto and Spoof (2005a)).

3.2.4. Coagulation/Flocculation/DAF Experiments

C/F/DAF experiments were performed in a laboratory-made flotation cell adapted from De Pinho *et al.* (2000). This apparatus (Figure 0.4) has a 2 L pressure chamber and a 3 L calibrated cylinder, where a paddle can be installed for the C/F prior to DAF.

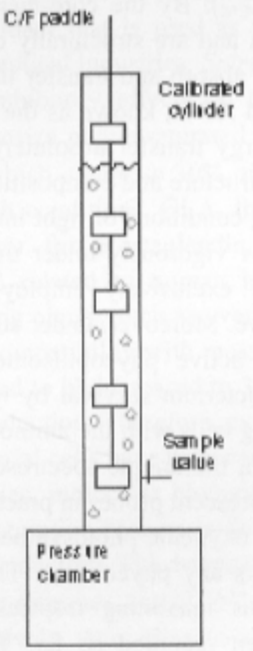

Figure 0.4. Scheme of the coagulation/flocculation/DAF *apparatus* (adapted from De Pinho *et al.* (2000)).

The experimental procedure for the C/F/DAF experiments followed Eckenfelder (2000) standard experimental procedure, but before the release of the recycle water volume, the paddle was installed in the cylinder, and a rapid (coagulation) and a slow (flocculation) mixing were performed. The operating conditions used corresponded to the ones that gave the best results in C/F/DAF experiments with *M. aeruginosa* single cells (Ribau Teixeira and Rosa (2006a)) namely: i) coagulation at a velocity gradient (G_C) of 380 s^{-1} for 2 min, using a pre-polymerised aluminium coagulant (aluminium polyhydroxichlorosulphate (WAC) with a relative basicity of 60-70%, Elf Atochem, stock solution with 850 mg/L Al_2O_3), the dose varying between 1–12 mg/L of Al_2O_3 depending on the type of water for *M. aeruginosa* cells

phycobiliproteins to form fluorescent antibody reagents; cyanophycin production; polyhydroxybutyrate biosynthesis; osmolytes production; nanoparticles formation; mosquito control; heavy metal removal; biodegradative ability of cyanobacteria; toxins formation by bloom-forming cyanobacteria; use of natural products of cyanobacteria for medicine and others aspects of cyanobacteria applications have been discussed in this chapter.

Chapter 7 - Cyanobacteria are unique in many ways and one unusual feature is the presence of a suite of proteins that contain at least one domain with a minimum of eight tandem repeated five-residues (Rfr) of the general consensus sequence A[N/D]LXX. The function of such pentapeptide repeat proteins (PRPs) are still unknown, however, their prevalence in cyanobacteria suggests that they may play some role in the unique biological activities of cyanobacteria. As part of an inter-disciplinary Membrane Biology Grand Challenge at the Environmental Molecular Sciences Laboratory (Pacific Northwest National Laboratory) and Washington University in St. Louis, the genome of *Cyanothece 51142* was sequenced and its molecular biology studied with relation to circadian rhythms. The genome of *Cyanothece* encodes for 35 proteins that contain at least one PRP domain. These proteins range in size from 105 (Cce_3102) to 930 (Cce_2929) amino acids with the PRP domains ranging in predicted size from 12 (Cce_1545) to 62 (cce_3979) tandem pentapeptide repeats. Transcriptomic studies with 29 out of the 35 genes showed that at least three of the PRPs in *Cyanothece 51142* (cce_0029, cce_3083, and cce_3272) oscillated with repeated periods of light and dark, further supporting a biological function for PRPs. Using X-ray diffraction crystallography, the structure for two pentapeptide repeat proteins from *Cyanothece 51142* were determined, cce_1272 (aka Rfr32) and cce_4529 (aka Rfr23). Analysis of their molecular structures suggests that all PRP may share the same structural motif, a novel type of right-handed quadrilateral □-helix, or Rfr-fold, reminiscent of a square tower with four distinct faces. Each pentapeptide repeat occupies one face of the Rfr-fold with four consecutive pentapeptide repeats completing a coil that, in turn, stack upon each other to form "protein skyscrapers". Details of the structural features of the Rfr-fold are reviewed here together with a discussion for the possible role of end-to-end aggregation in PRPs.

Chapter 8 - Cyanobacteria (blue-green algae) are ancient photosynthetic prokaryotes which inhabit a wide range of terrestrial and aquatic environments. Under certain aquatic conditions, they are able to proliferate to form extensive blooms, scums and mats, particularly in nutrient-rich waters which may be used for the preparation of drinking water and for recreation, fisheries and crop irrigation. Although not pathogens, many cyanobacteria can produce a wide range of toxic compounds (cyanotoxins) which act through a variety of molecular mechanisms. Cyanotoxins are predominantly characterised as hepatotoxins, neurotoxins and irritant toxins, and further bioactive cyanobacterial metabolites, with both harmful and beneficial properties, are emerging. Human and animal poisoning episodes have been documented and attributed to cyanotoxins, ranging from the deaths of haemodialysis patients in Brazil to a wide range of animal species, including cattle, sheep, dogs, fish and birds. Some purified cyanotoxins are classified as Scheduled Chemical Weapons as they are among the most toxic naturally-occurring compounds currently known and several countries have introduced Anti-Terrorism Legislation to monitor the use and supply of certain purified cyanobacterial toxins. A wide range of physico-chemical and biological methods is available to analyse the toxins and genes involved in their synthesis, which may be applicable to monitoring aspects of cyanobacteria and bioterrorism.

Chapter 9 - Available freshwater resources are polluted by industrial effluents, domestic and commercial sewage, as well as mine drainage, agricultural run-off and litter. Among water pollutants, heavy metals are priority toxicants that pose potential risks to human health and the environment. Bacterial bioreporters may complement physical and chemical analytical methods by detecting the bioavailable (potentially hazardous to biological systems) fraction of metals in environmental samples. Most bacterial bioreporters are based on heterotrophic organisms; cyanobacteria, although important primary producers in aquatic ecosystems, are clearly underrepresented. In this chapter, the potential use of self-luminescent cyanobacterial strains for ecotoxicity testing in aqueous samples has been evaluated; for this purpose, a self-luminescent strain of the freshwater cyanobacterium *Anabaena* sp. PCC 7120 which bears in the chromosome a Tn5 derivative with *luxCDABE* from the luminescent terrestrial bacterium *Photorhabdus luminescens* (formerly *Xenorhabdus luminescens*) and shows a high constitutive luminescence has been used. The ecotoxicity assay that has been developed is based on the inhibition of bioluminescence caused by biologically available toxic compounds; as a toxicity value, authors have used the effective concentration of each tested compound needed to reduce bioluminescence by 50% from that of the control (EC_{50}). The bioassay allowed for acute as well as chronic toxicity testing. Cyanobacterial bioluminescence responded sensitively to a wide range of metals; furthermore, the sensitivity of the cyanobacterial bioreporter was competitive with that of published bacterial bioreporters. In contaminated environments, organisms are usually exposed to a mixture of pollutants rather than single pollutants. The toxicity of composite mixtures of metals using the cyanobacterial bioreporter was tested; to understand the toxicity of metal interactions, the combination index CI-isobologram equation, a widely used method for analysis of drug interactions that allows computerized quantitation of synergism, additive effect and antagonism has been used. Finally, this study indicates that cyanobacterial-based bioreporters may be useful tools for ecotoxicity testing in contaminated environments and that the CI-Isobologram equation can be applied to understand the toxicity of complex mixtures of contaminants in environmental samples.

Chapter 10 - Microbial mats consist of multi-layered microbial communities organized in space as a result of steep physicochemical gradients. They can be found in sheltered and shallow coastal areas and intertidal zones where they flourish whenever extreme temperatures, dryness or saltiness act to exclude plants and animals. Several metabolically active microorganisms, such as phototrophs (i.e., diatoms, cyanobacteria, purple and green sulfur bacteria) develop in microbial mats together with chemoautotrophic and heterotrophic bacteria.

These communities have been observed to grow in polluted sites where their ability to degrade petroleum components has been demonstrated. Furthermore, several investigations have attributed to cyanobacteria an important role in the biodegradation of organic pollutants. Nevertheless, it is still a matter of discussion whether cyanobacteria can develop using crude oil as the sole carbon source. In an attempt to evaluate their role in hydrocarbon degradation authors have developed an illuminated packed tubular reactor filled with perlite soaked with crude oil inoculated with samples from Ebro Delta microbial mats. A continuous stream of nutrient-containing water was circulated through the system. Crude oil was the only carbon source and the reactor did not contain inorganic carbon. Oxygen tension was kept low in order to minimize possible growth of cyanobacteria at the expense of CO_2 produced from the degradation of oil by heterotrophic bacteria. Different microorganisms were able to develop

attached to the surface of the filling material, and analysis of microbial diversity within the reactor using culture-independent molecular techniques revealed the existence of complex assemblages of bacteria diverse both taxonomically and functionally, but cyanobacteria were not among them. However, cyanobacteria did grow in parallel oil-containing reactors in the presence of carbonate.

Chapter 11 - In cyanobacteria, bioluminescence reporters have been applied to the measurement of physiological phenomenon, such as in the study of circadian clock and nitrite, ferric, and light responses. Cyanobacterial researchers have so far used several types of bioluminescence reporter systems—consisting of luminescence genes, genetically tractable host cells, and a monitoring device—because their studies require a method that offers gene expression data with high fidelity, high resolution for time, and enough dynamic range in data collection. In addition, no extraction of the products of the reporter gene from the culture is required to measure the luminescence, even in the living cell. In this chapter, applications using the bioluminescence genes *luxAB* (and *luxCDE* for substrate production) and insect genes are introduced. For measurement and imaging, general apparatuses, such as a luminometer and a luminoimager, have been used with several methods of substrate administration. Automated bioluminescence monitoring apparatuses were also newly developed. The initial machine was similar to that used to measure the native circadian rhythms in bioluminescence of the marine dinoflagellate *Gonyaulax polyedra*. Then, the machine with a cooled CCD camera which was automatically operated by a computer was used to screen mutant colonies representing abnormal bioluminescence profile or level from a mutagen-treated cyanobacterial cell with a *luxAB* reporter. Recently, different two promoter activities could be examined in the same cell culture and with the same timing by using railroad-worm luciferase genes. The bioluminescence rhythm monitoring technology of the living single-cell in micro chamber was developed. These might expand authors knowledge to understand other cyanobacterial fields and microorganisms. Here, authors provide a guide on the genes, the targeting loci in the genome, the apparatus and machines, and the studies utilizing the bioluminescence.

Chapter 12 - The human heath risk potential associated with the presence of cyanobacteria and cyanotoxins in water for human consumption has been evaluated. This risk is related to the potential production of taste and odour compounds and toxins by cyanobacteria, which may cause severe liver damage, neuromuscular blocking and are tumour promoters. Therefore, its presence in water, used for drinking water production and/or recreational activities, even at low concentrations, has particular interest to the water managers due to the acute toxicity and sublethal toxicity of these toxins, and may result in necessity of upgrading the water treatment sequences.

The need for risk management strategies to minimize these problems has been recognised in different countries. One of these strategies could pass through the implementation of a safe treatment sequence that guarantees a good drinking water quality, removing both cyanobacteria and cyanotoxins, despite prevention principle should be the first applied.

This work is a contribution for the development of one of these sequences, based on the removal of intact cyanobacteria and cyanotoxins from drinking water, minimising (or even eliminating) their potential heath risk. The sequence proposed is dissolved air flotation (DAF) and nanofiltration: DAF should profit the flotation ability of cyanobacteria and remove them without cell lysis, *i.e.* without releasing the cyanotoxins into the water; nanofiltration should

remove the cyanotoxins present in water (by natural and/or induced release) down to a safe level for human supply.

Results indicated that DAF – nanofiltration sequence guaranteed a full removal of the cyanobacterial biomass (100% removal of chlorophyll *a*) and the associated microcystins. Microcystin concentrations in the treated water were always under the quantification limit, *i.e.* far below the World Health Organization (WHO) guideline value of 1 µg/L for microcystin-LR in drinking water. Therefore, this sequence is a safe barrier against *M. aeruginosa* and the associated microcystins variants in drinking water, even when high concentrations are present in raw water, and nanofiltration water recovery rates as high as 84% could be used. In addition, it ensures an excellent control of particles (turbidity), and disinfection by-products formation (very low values of DOC, UV_{254nm} and SUVA were achieved), as well as other micropollutants (above *ca.* 200 g/mol, *e.g.* anatoxin-a) that might be present in the water.

Chapter 13 - Cyanobacteria grow by photosynthesis, and essentially contain chlorophyll and various carotenoids whose main functions are light-harvesting and photoprotection. In this chapter, authors have summarized carotenoids, characteristics of carotenogenesis enzymes and genes, and carotenogenesis pathways in some cyanobacteria, whose carotenoids and genome DNA sequences have both been determined. Cyanobacteria contain various carotenoids: β-carotene, its hydroxyl or keto derivatives, and carotenoid glycosides. Both ketocarotenoids, such as echinenone and 4-ketomyxol, and the carotenoid glycosides, such as myxol glycosides and oscillol diglycoside, are unique carotenoids in phototrophic organisms. Some cyanobacteria contain both unique carotenoids, while others do not contain such carotenoids. From these findings, certain carotenogenesis pathways can be proposed. The different compositions of carotenoids might be due to the presence or absence of certain gene(s), or to different enzyme characteristics. For instance, two distinct β-carotene hydroxylases, CrtR and CrtG, are bifunctional enzymes whose substrates are both β-carotene and deoxymyxol, and substrate specificities of CrtR vary across species. Two distinct β-carotene ketolases, CrtO and CrtW, are found only in the first group and properly used in two pathways, β-carotene and myxol, depending on the species. At present, the number of functionally confirmed genes is limited, and only a few species are examined. Therefore, further studies of carotenoids, characteristics of carotenogenesis enzymes and genes, and carotenogenesis pathways are needed.

Chapter 14 - Cyanobacteria are renowned for the biosynthesis of a range of natural products. In comparison to the bioactives produced by non-ribosomal peptide synthetase and polyketide synthase systems, the hapalindole family of hybrid isoprenoid-indole alkaloids has received considerably less attention. It has been proposed that these natural products, the indole alkaloids, are constructed by a pathway of monofunctional enzymes. This chapter will specifically discuss the hapalindole family of alkaloids isolated exclusively from the Group 5 cyanobacteria. Structural diversity within this family correlates with a wide range of bioactivities. However, despite the wide variety of structures related to the hapalindoles, their biosynthesis is proposed to occur via a common pathway. Structural diversification of the natural products is proposed to have occurred as a result of evolution of biosynthetic enzymes in Nature and thus will provide insights into how these and related enzymes may be engineered in the laboratory. In this chapter authors will focus on aspects of hapalindole

structural diversity, proposed biosynthetic pathways, known bioactivities, and the potential for bioengineering of this unique natural product class.

Chapter 15 - This paper briefly examines the future prospects for the economical viability of large-scale renewable energy production using maricultured cyanobacteria. In order to reduce CO_2 emissions from burning fossil fuels in appreciable amounts, the replacement energy source will by necessity be substantial in scale. Solar energy is the most likely candidate because the amount of solar energy received on the Earth's surface is vast and exceeds the anthropogenic primary energy use by more than 6,000 times. Although solar energy is abundant, its economical utilization is not straightforward because the intensity received on the surface of the earth is relatively low. Current research and development efforts are focused on the production of biofuels as renewable, economical feasible energy sources from the land biomass. The authors propose, however, for reasons of scale and to minimize further environmental harm, that the utilization of the sea surface is a more viable alternative to land biomass exploitation. The sea surface area available for energy production far exceeds available cropland and use of the sea will not take valuable cropland out of food production. The authors current R & D strategy utilizes photosynthesis and the nitrogenase enzyme of cyanobacteria. The biological basis of relevant energy metabolism in cyanobacteria is briefly described. A model for future H_2 production systems is presented, and a very rough trial calculation of the cost of photobiological H_2 production is made in the hope that it may help the readers recognize the possibilities of large-scale H_2 production and understand the need for the research and development.

Chapter 16 - Marine microbial symbionts represent a hotspot in the field of marine microbiology. Marine plants and animals, such as sponge, sea squirt, worm, and algae host symbiotic cyanobacteria with great diversity. Most of the symbiotic cyanobacteria are host-specific and can be transmitted directly from parent to offspring. Symbiotic cyanobacteria play an important role in nitrogen fixation, nutrition and energy transfer and are possible true producers of bioactive marine natural products. Though diverse cyanobacteria have been revealed by culture-independent methods, the isolation and culture of symbiotic cyanobacteria is a challenge. In this chapter, the advances in diversity, transmission, symbiotic relationship with the host, isolation and natural products of marine symbiotic cyanobacteria are reviewed.

Chapter 17 - Previous research has discovered that pesticides which generate reactive oxygen species (ROS), such as the bipyridilium herbicides diquat and paraquat, and certain natural compounds (e.g., quinones) are selectively toxic towards undesirable species of cyanobacteria (blue-green algae) (division Cyanophyta) compared to preferred green algae (division Chlorophyta) commonly found in channel catfish (*Ictalurus punctatus*) aquaculture ponds. In this study, the antioxidant enzyme activities of the green alga *Selenastrum capricornutum* and the cyanobacteria *Planktothrix agardhii*, *Planktothrix perornata*, and *Raphidiopsis brookii*, previously isolated from catfish aquaculture ponds in west Mississippi, were measured to help determine the cause for the selective toxicity of ROS-generating compounds. Enzyme assays were performed using cells from separate continuous culture systems to quantify and correlate the specific enzyme activities of superoxide dismutase, catalase, ascorbate peroxidase, and glutathione peroxidase relative to the protein content of the cells. The cyanobacteria used in this study have significantly lower specific activities of superoxide dismutase, catalase, and ascorbate peroxidase when compared to *S. capricornutum*. Glutathione peroxidase activity was not detected in these cyanobacteria or *S.*

capricornutum. The deficiency of measured antioxidant enzyme activities in the test cyanobacteria is at least one reason for the selective toxicity of ROS-generating compounds towards these cyanobacteria compared to *S. capricornutum*.

Chapter 18 - Cyanobacteria produce numerous bioactive compounds including vitamin B_{12}. Corrinoid compound found in various edible cyanobacteria (*Spirulina* sp., *Nostoc* sp., *Aphanizomenon* sp., and so on) were identified as pseudovitamin B_{12} (*7*-adeninyl cobamide), which is inactive for humans. Edible cyanobacteria are not suitable for use as a vitamin B_{12} source, especially in vegetarians.

Analysis of genomic information suggests that most cyanobacteria can synthesize the corrin ring, but not the *5,6*-dimethylbenzimidazolyl nucleotide moiety in vitamin B_{12} molecule. Therefore, the bacterial cells would construct a corrinoid compound as pseudovitamin B_{12} by using a cellular metabolite, adenine nucleotide. Pseudovitamin B_{12} appears to function as coenzymes of cobalamin-dependent methionine synthase or ribonucleotide reductase (or both).