BOOK OF ABSTRACTS

Green Christmas Session

PHOTOSYNTHETIC MICROORGANISMS FOR SUSTAINABLE DEVELOPMENT

16 - 17 December 2021

Rome, Italy

CONFERENCE PROGRAM AND ABSTRACTS

GREEN CHRISTMAS SESSION

"PHOTOSYNTHETIC MICROORGANISMS FOR SUSTAINABLE DEVELOPMENT"

INTERNATIONAL ONLINE CONFERENCE

TOPICS:

Green energy: light-harvesting, bioelectricity and biofuels

Space research

Production of compounds with high-added value

Sustainability and environment

conference web page: http://www.isb.cnr.it/test2/index.php/gcs-2021/

free registration at https://forms.gle/MQhhBPkLDZYrohwH9

contact us at gcs.org@cnr.it

16-17 December 2021, Rome, Italy

The **Green Christmas Session** has been thought of as a series of interactive online meetings to be organized annually. The discussions will be focused on different fundamental and applied aspects of photosynthesis research and on its potential to propose sustainable solutions in resolving emerging questions and problems of modern society.

The Green Christmas Session 2021 is under the patronage of the Italian Society of Photobiology (ISFB) and the Russian Society for Photobiology (RSP)

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The Conference is hosted by the Institute for Biological Systems, National Research Council, AdR Roma 1

WELCOME

Dear participants,

We are glad to welcome you to the first online Green Christmas Session (GCS) "Photosynthetic microorganisms for sustainable development". The GCS 2021 will showcase the potential of the photosynthetic microorganisms to take an active part in the elaboration of sustainable approaches to support the development of modern society.

Oxygenic photosynthesis is the most essential biological process for the maintenance of life on Earth. Understanding the photosynthetic mechanisms allows us to raise the level of acquaintance with the world around us and to achieve significant successes in satisfying human needs at minimum Nature expense. Photosynthetic microorganisms, such as microalgae and photosynthetic bacteria, are of great importance as organisms with high photosynthetic conversion efficiency, an extraordinary capacity to adapt to different growth environments and unique metabolic diversity and flexibility. The scientific knowledge on the mechanisms of primary photochemistry and environmental adaptation might be applied for solar-powered energy generation, for environmental protection, for the development of life-support systems in manned Space missions and the production of a plethora of high-added value compounds.

The main purpose of the GCS 2021 is to provide a platform for discussing the latest trends and advancements in the development of bio-based applications employing photosynthetic microorganisms or their photosynthetically active pigment-protein assembles. Although the event is keen to deepen the collaboration between the photosynthesis research communities of Italy and Russia, it will bring together scholars, early-stage researchers and students from all around the world. The format of the conference envisages the participation of Invited Speakers and free attendance for the audience, and foresees time for Q&A after the individual presentations.

Again, welcome and experience a fruitful exchange of knowledge!

Maya Dimova Lambreva	Maria Borisova-Mubarakshina
(GCS chair)	(GCS co-chair)

CONFERENCE PROGRAM

The conference schedule is set to Central European Time (+2h Moscow Standard Time)

16th December 2021

09:00 Online Platform Opening

09:10 Welcome address by **Giovanna Mancini**
President of the Area of Research Rome1 and Director of the Institute for Biological Systems, CNR

Welcome address by **Dariya Pushkova**
Director of the Russian Centre for Science and Culture in Rome

SESSION: Green energy: light-harvesting, bioelectricity and biofuels

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12:50	**Taras Antal** (*Pskov State University, Pskov*) *Assessment of ecological state of natural water bodies*	Page 20
13:10	**Round table & Closing remarks**	

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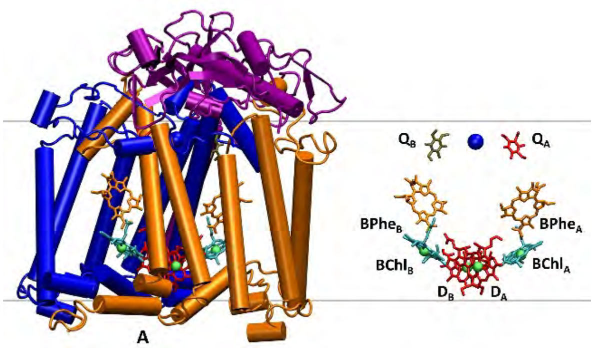
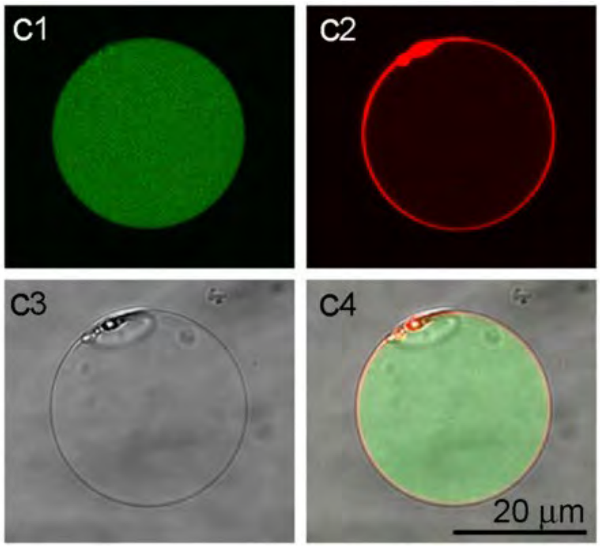
Photosynthesis for a sustainable future

Keywords: photosynthesis, energy, synthetic biology

Planet Earth sustains all its life forms by exploiting photosynthesis, a paramount biological process performed by plants, algae, and some bacteria. Photosynthesis is the sole biological process able to fix energy on Earth by harvesting sunlight. The planetary relevance of such metabolic process was clear to Giacomo Ciamician, one of the founders of modern photochemistry who delivered a visionary in 1912 speech on the urgency of substituting fossil fuels with "The enormous quantity of energy that the Earth receives from the sun", anticipating by more than one century what is now an almost universally shared opinion.
Eventually, spurred by the vision of Ciamician, at the end of the last century the idea of artificial photosynthesis for producing sustainable energy kicked in the scientific community. More recently, the possibility to use whole, metabolically active photosynthetic organisms in technological applications is gaining momentum in the scientific community. The recent attempts made in our laboratory to harness light and exploit it are a useful little roadmap to employ photosynthetic bacteria in environmental applications.





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Photosystem II in biohybrid devices for solar energy conversion

Keywords: artificial photosynthesis, photosystem II, solar cell, alternative energy

The use of traditional energy sources has given rise to several ecological challenges that pursued scientists to study and develop the alternative ways to obtain energy. Among the renewable energy sources, solar light is the most accessible and abundant, and it can be effectively converted to energy via photosynthetic components with a quantum yield close to 100%. Therefore, photosynthetic complexes are reasonable to consider as photosensitizers for the application in artificial photosynthesis. As a part of biohybrid devises, the components of photosynthetic apparatus from higher plants, microalgae, and cyanobacteria can be used to generate photocurrent. In order to design biohybrid solar cell, the photoelectrode with immobilized photosynthetic complexes should be developed. Recently, isolated photosystem I (PSI) and II (PSII) are considered to be promising components for photocurrent generation due to their smaller size comparing to thylakoid membranes or the whole cells. The small size of the photosensitizer allows to increase the number of immobilized complexes and, ultimately, to improve the efficiency of the cell. However, PSI requires the addition of exogenous electron donor with a redox potential equal to that of natural donor plastocyanin. In this case, PSII has the advantage as the water abundant in nature serves as an electron donor and is oxidized by PSII water oxidizing complex. Nevertheless, the main problem of biohybrid system of energy generation lays in low stability of isolated natural components. This challenge still needs to be overcome in construction of such artificial photosynthesis systems, and several attempts have been already made.

The work has been supported by Russian Scientific Foundation, project 19-14-00118.

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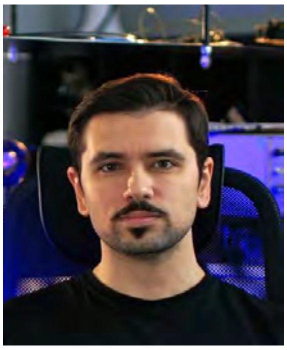

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Design of hybrid photoactive proteins

Keywords: orange carotenoid protein, fluorescence, photoprotection

Here, we propose a possible photoactivation mechanism of a 35-kDa blue light-triggered photoreceptor, the Orange Carotenoid Protein (OCP), suggesting that the reaction involves the transient formation of a protonated ketocarotenoid (oxocarbenium cation) state. Taking advantage of engineering an OCP variant carrying the Y201W mutation, which shows superior spectroscopic and structural properties, it is shown that the presence of Trp201 augments the impact of one critical H-bond between the ketocarotenoid and the protein. This confers an unprecedented homogeneity of the dark-adapted OCP state and substantially increases the yield of the excited photoproduct S*, which is important for the productive photocycle to proceed. A 1.37 Å crystal structure of OCP Y201W combined with femtosecond time-resolved absorption spectroscopy, kinetic analysis, and deconvolution of the spectral intermediates, as well as extensive quantum chemical calculations incorporating the effect of the local electric field, highlighted the role of charge-transfer states during OCP photoconversion.

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Analysing the energetic interactions of photosynthetic systems with carbon nanotubes

Keywords: carbon nanotubes, photosynthetic complexes, electron transfer, excitation decay

The fusion of highly dynamic and adaptive photosynthetic structures with easily manipulated inorganic materials at nanoscale level might pave the way for new opportunities to develop solar-powered biotechnology for renewable energy production. It was suggested that single-walled Carbon NanoTubes (CNTs) may increase plant photosynthesis *in vitro* and *in situ* by transferring energy to photosynthetic reactions (Nat.Mater.2014,13:400-408). The mechanism of this phenomenon is still unclear and the limited number of studies dealing with the CNT interplay with PhotoSynthetic Complexes (PSCs) provide controversial indications about the energy flow within this biohybrid system. Our studies of the CNT effects on the photochemical reactions in *Chlamydomonas reinhardtii* pointed out that the nanotubes can alter the electron transport of photosystem II and may facilitate the non-radiative loss of excitation energy in antenna complexes. To obtain further insights into these processes, we analysed the interactions of CNTs with isolated PSCs and supramolecular assemblies with different levels of complexity. The possible routes involved in the energy excitation decay in the photosynthetic structures in the studied model systems will be discussed.

This work was funded by STM 2018 program of CNR and CNR-RFBR joint research project Lambreva/Rubin 2015-2017.

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Bio-hybrid electrochemical systems for energy and the environment

Keywords: microbial electrochemistry, semi-artificial photosynthesis, biosensors

Various microorganisms and organelles are capable of transferring (and uptake) electrons to (from) electrode surfaces, a process defined as extracellular electron transfer.[1] This unique capability paved the way to the development of bio-hybrid electrochemical systems for sustainable micro/low power production, biosensing, and bioelectrosynthesis. Utilizing photosynthetic entities enables converting solar energy into electrical energy; however, the photosynthetic apparatus in intact biocatalysts (i.e., chloroplasts, purple bacteria, and cyanobacteria) is physically separated from the electrode surface by the presence of various membrane layers, hindering the electron transfer. As a result, research efforts have been focused on developing artificial approaches to facilitate the transfer of photoexcited electrons from these photosynthetic entities to the electrodes (and vice versa).[2] Herein, practical approaches for accomplishing the photoexcited extracellular electron transfer will be presented, together with the applications of the obtained biohybrid electrochemical systems for environmental monitoring.[3] Finally, enthralling future research directions will be discussed, with the possible application of purple bacteria in bio-hybrid electrochemical systems for metal ions monitoring.[4]

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3. M. Grattieri et al. *Chem. Commun.* 2020, **56**, 13161-13164.
4. M. Grattieri et al. *Photochem. Photobiol. Sci.* 2021 DOI:10.1007/s43630-021-00116-9.

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Cytosolic NiFe-hydrogenases: structure, functions, possibilities of practical application

Keywords: hydrogenases, biohydrogen, NADH/NADPH, purple sulfur bacteria, cyanobacteria

Hydrogenases are the main enzymes of hydrogen metabolism. They are present in bacteria, archaea, microalgae and protists. They can be divided into three main classes based on the metal ions in active centers or cofactors, namely Fe-, FeFe- and NiFe-hydrogenases. NiFe hydrogenases are a diverse group of enzymes specific for prokaryotes, they can be localized in plasma membrane and in cytosol. According to HydDB hydrogenase classifier, so-called bidirectional cytosolic hydrogenases belong to group 3 of NiFe hydrogenases. Subgroups 3a and 3c are coenzyme F420-dependent and heterodisulfide reductase-linked enzymes, respectively; subgroups 3b and 3d are NADP- and NAD-coupled enzymes. Three-dimensional structures are known for hydrogenases from subgroups 3c (PDB ID: 5ODC) and 3d (PDB IDs: 5XF9, 5XFA). NAD- and NADP-dependent hydrogenases are present in such phototrophic organisms as cyanobacteria, purple sulfur bacteria (*Chromatiaceae*), green sulfur bacteria (*Chloroflexi*). The present work is dedicated to structural modeling of hydrogenases from these photosynthetic organisms and an attempt to find out the differences between NADP- and NAD-dependent enzymes. Biotechnological potential of cytosolic hydrogenases is discussed, their three advantages compared to other subgroups are taken into account: 1) the ability to take electrons from naturally abundant electron donors (NADPH, NADH, ferredoxin); 2) the possibility of heterologous expression of these enzymes; 3) the simplicity of "minimal hydrogenase" that could be derived from a full cytosolic hydrogenase complex via deletion of diaphorase component.

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Desert cyanobacteria and *in situ* resource utilization to support human space exploration

Keywords: cyanobacteria, space exploration, moon, mars, life support

Sustaining human outposts on Mars by providing life-support consumables from Earth is unrealistic mainly due to launch costs, travel times and failure risks. The development of Life Support Systems (LSS) is mandatory. An important aspect of LSS is the utilization of local materials, the so-called in-situ resource utilization (ISRU), although, to date, these technologies focused mainly on inorganic chemistry and building materials. Cyanobacterial adaptation to hot and cold deserts and their resistance when dried to space and Mars-like conditions, make them promising components of future life-support systems. Moreover, their lithotrophic capability is a key feature for their growth on Mars or Moon regoliths, thus allowing the link between local resources and LSS, otherwise unable to live off the land. As a proof-of-concept the space-relevant desert *Chroococcidiopsis* sp. CCMEE 029 was grown on Mars and Moon regolith simulants and its lysate used to feed the heterotrophic bacterium *Escherichia coli*.

This research is carried out in the context of the ASI-funded project ReBUS – In-situ Resource Bio-Utilization for Life Support (grant n° 2019-4-U.0).

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The effect of high-energy charged particles on primary processes of energy conversion in the photosynthetic apparatus of cyanobacteria

Keywords: space, cosmic rays, ionizing radiation, cyanobacteria, photosynthesis, phycobilisomes, phycobiliproteins, orange carotenoid protein, carotenoids, reactive oxygen species

In the current work, a comparative study of the influence of space flight conditions and model experiments on the photosynthetic apparatus of cyanobacterium Synechocystis sp. PCC 6803 (Synechocystis) was presented. The cell culture of the wild-type Synechocystis launched into space on the satellite “Foton M4”, and it orbited the Earth for 45 days. For modelling conditions of the space flight, cyclotron U-120 SINP MSU was used, which yields accelerated helium nuclei with an energy of about 30.3 MeV. Changes in the structural and functional state of the photosynthetic membranes of cyanobacteria were obtained by a variety of optical methods, including fluorescence spectroscopy with picosecond time resolution, and modelling the thylakoid membranes. Phycobilisomes decouple from the photosystems under exposure to ionizing radiation. A possible radioprotective role of the phycobilisomes was revealed. After space flight or exposure to high doses of ionizing radiation, the cells of Synechocystis sp. remained capable of autotrophic growth and were able to restore cell culture with spectral and functional characteristics indistinguishable from control samples. The cell culture of Synechocystis sp., which returned to Earth after space flight, was included in the collection of microorganisms of the Faculty of Biology, Lomonosov Moscow State University.

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Assessing cyanobacteria photosynthetic responses under red dwarfs simulated spectra

Keywords: red dwarfs, cyanobacteria, photosynthesis

Abstract Several recently discovered Earth-like exoplanets are orbiting the Habitable Zone of M-dwarf stars. These are the most abundant and long-lived stars known in the Milky Way, but have different spectral characteristics respect to the Sun. They are less luminous and generate a light spectrum with far-red and infrared as major components, while emitting at very low level in the visible. These characteristics do not seem suitable for most oxygenic photosynthetic organisms we know from Earth, that evolved to absorb only visible light. Many researchers discussed the possibility of oxygenic photosynthesis in these worlds so far, but no experimental research has been done testing organisms under simulated M-dwarf spectra. At the university of Padova, a collaboration between the Department of Biology, the Astronomical Observatory (INAF) and the Institute of Photonics and Nanotechnology (IFN-CNR) led to the construction and the development of a new experimental tool. The setup is composed by three main parts: 1) a Star Light Simulator, able to generate different light intensities and spectra, including those of nonsolar stars; 2) an Atmosphere Simulator Chamber where cultures of photosynthetic microorganisms can be exposed to different gas compositions; 3) a reflectivity detection system to measure from remote the Normalized Difference Vegetation Indexes (NDVI). Such a setup allows us to monitor photosynthetic microorganism's growth and gas exchange performances under selected conditions of light quality and intensity, temperature, and atmospheres simulating non-terrestrial environments.

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Microalgal biotechnology and sustainable usage of phosphorus

Keywords: microalgae, luxury phosphorus uptake, polyphosphate, biofertilizer

Microalgae are believed to evolve under conditions of scarce and fluctuating availability of phosphorus (P). Adaptations to P shortage include rearrangements of lipid metabolism and polar metabolome, downregulation of photosynthetic activity, and luxury uptake of P. The latter is defined as taking up more P that necessary for the progression of current cell cycle. Luxury uptake of P is the basis of the nutrient conversion from waste streams to phosphorus biofertilizers. The mechanisms of microalgae acclimation to P starvation are relatively well studied, but luxury uptake of P remains enigmatic in many regards. Phosphorus starvation induces smaller penalties for the culture growth e.g. in comparison with those of nitrogen starvation since nutrient-replete microalgal cells normally possesses a sizeable reserve of P, frequently in the form of inorganic polyphosphates which are created during the periods of luxury P uptake. Nevertheless, high concentrations of exogenic P can be toxic resulting in the inhibition of microalgal cell division or even death which can be a concern for wastewater biotreatment with microalgal cultures. We hypothesize that P toxicity arises when the rate of P uptake is much faster than its conversion into long-chain polyphosphate coupled with their transport into the cell vacuole(s). Instead of this, a lot of short-chain polyphosphate molecules chaotically distributed in the cytoplasm are formed disturbing with protein folding. This can be the case during re-feeding of P-starved cultures with large amounts of P.

Understanding the relationships between P availability, cell viability and capacity for P uptake is essential for the development of efficient knowledge-based microalgal biotechnologies both for valuable substance production and protection of the environment.

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Metabolic engineering of microalgae photosynthesis to increase productivity in photobioreactors

Keywords: microalgae, photosynthesis, metabolic engineering, photobioreactors, models

Microalgae biomass is a promising solution to convert current industrial processes for the production of basic bio-commodities to sustainable alternatives.
In this scenario, microalgae must be cultivated in large photobioreactors or ponds to fulfil the demand of biomass on a global scale, but this artificial environment is far different from the natural niches these organisms evolved in. In industrial cultivation conditions, microalgae are in fact exposed to a broad spectrum of natural and artificial environmental changes that influence photosynthesis, all with a strong detrimental impact on biomass productivity.
Such complex environment calls for genetic strategies to optimise the photosynthetic metabolism and improve biomass productivity to concretize the development of a microalgae-biomass-based economy. We have optimized several features of microalgae photosynthesis so far, showing substantial improvement in biomass productivity at the lab-scale and proving domestication is a required step before microalgae cultivation reaches economic feasibility at industrial scale.
As domesticated crops must have improved performances on the field, optimized microalgae strains must show improved productivity in photobioreactors. This environment is far different from the lab-scale conditions in which domesticated strains are being isolated and tested, and here improved performances cannot be predicted in advance. We observed that microalgae photosynthesis acclimates to the environment of photobioreactors but this phenomenon is still poorly understood, often limiting the achievements we can obtain through metabolic engineering. Mathematical models might represent the connecting line between lab and industrial scale, enabling a more effective design of metabolic engineering strategies in the near future.

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Keto-carotenoids production in model cyanobacteria

Keywords: keto-carotenoids; metabolic engineering; Synechocystis sp. PCC 6803.

Cyanobacteria are widely distributed in marine, freshwater and terrestrial environments, constituting the most important primary producers on our planet. Their outstanding success in colonising almost any environment on Earth is due to their simple growth requirements and metabolic plasticity. Moreover, thanks to the availability of several genetic and synthetic biological tools for their manipulation, cyanobacteria are receiving considerable attention as promising alternative sources for the production of compounds of industrial interest. By introducing the carotenogenic genes *CrtW* and *CrtZ* of the proteobacterium *Brevundimonas* sp. SD-212 in the model species *Synechocystis* sp. PCC 6803, we generated engineered strains able to accumulate, constitutively or in a temperature-inducible manner, the valuable keto-carotenoids cantaxanthin and astaxanthin, which are largely employed in the food, feed, nutraceutical, cosmetic and pharmaceutical sectors. Limits and perspectives of the two engineering approaches will be discussed, together with the challenges faced by scaling up the process as well as the possibility to exploit other cyanobacterial species for the sustainable production of non-endogenous keto-carotenoids.

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High-added-value compounds from microalgae

Keywords: microalgae, cyanobacteria, fatty acids, lipids, carbohydrates, carotenoids

Microalgae are microscopic organisms, which are basically oxygenic photoautotrophs. Microalgae are phylogenetically diverse and differ in their biochemistry: they are capable of synthesizing different types of carbohydrates, lipids with high variety of fatty acids, pigments of various types, vitamins, sterols and secondary metabolites including antibiotics and toxins. Due to unique metabolic plasticity of microalgae, it is possible to enrich their biomass with proteins, carbohydrates, lipids or pigments by changing growth conditions. We investigated the effect of salt stress on eustigmatophycean microalga *Vischeria punctata* strain IPPAS H-242, a superproducer of eicosapentaenoic acid (EPA). We found that under salt stress the growth was inhibited; cells were bigger, with higher chloroplast volume and numerous mitochondria, they had more proteins and their lipids had higher EPA proportion in total FA. However, the total EPA productivity decreased under the salt stress. Also we studied the dynamics of the accumulation of starch and triacylglycerols (TAG) in three strains of green microalgae (*Chlorella* sp. IPPAS C-1210, *Coelastrella* sp. IPPAS H-626, *Nannochloris* sp. IPPAS C-1509) during growth in a batch culture in full medium or under conditions of various elements starvation (N, P, Mg, S, Fe). We found that Mg starvation caused carbohydrate accumulation, while N starvation is most effective trigger for TAG accumulation. The deep transcriptomes of the green microalgae strains were studied and main metabolic genes were identified.

The work was supported by Russian Science Foundation grant [no. 20-14-00280].

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Purple non-sulphur bacteria as a source of bioplastics

Keywords: purple non-sulphur bacteria, photobioreactor, polyhydroxyalkanoates, poly-3-hydroxybutyrate.

Photosynthetic production of energy and organic materials addresses global warming and environmental pollution. Photosynthetic microorganisms such as purple non-sulphur bacteria (PNSB) are currently being investigated as potential source of biomaterials and biofuels. They are fast-growing organisms, capable of achieving high biomass productivities and mainly use sunlight as the energy source. They are promising candidates for bioplastic, such as polyhydroxyalkanoates and biohydrogen production. Polyhydroxyalkanoates are mainly accumulated in the cytoplasm of PNSB, as carbon and energy storage molecules, when they grow under stressful conditions. The deployment of PNSB as a reservoir for bio-products requires the use of efficient photobioreactors (PBRs). PBRs must be designed to have an optimal use of light and suitable mixing to ensure homogeneous illumination of the culture. This work presents experimental results concerning PNSB growth in different types of photobioreactors for the poly-3-hydroxybutyrate production using synthetic culture broths and low-cost agro-industrial wastewaters. The results indicated the fact that cell growth and poly-3-hydroxybutyrate production were affected by the type of carbon source in the culture broth.

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Microalgae polysaccharides: a novel bio-material for cultural heritage preservation

Keywords: microalgae, polysaccharides, cultural heritage, sustainability, novel material

Cultural heritage, whatever its composition and location, is susceptible to deterioration. One of the main challenge in this sector is the promotion of innovative products for the restoration/protection of artistic value surfaces. In this context, we propose the development of a new product polysaccharides-based, extracted from microalgae, compatible with ancient materials, not harmful for humans and environment. The qualities of algal polysaccharides have been described for their significant antioxidant, antifungal, cosmeceutical and nutraceutical activities in medical, agri-food and cosmetics fields, but still little is known in cultural heritage sector. Hence, we utilize the polysaccharidic extracts to foster an innovative restoration and conservation of artistic work, promoting mechanical renewal, as well as hindrance the onset of biotic colonization. For these reasons, specific microalgae were selected and grown to induce a natural accumulation of carbohydrates. Furthermore, an extraction protocol for polysaccharide mixture was optimized and its composition determined by FT-IR, NMR, elemental chemical analysis, as well as its antioxidant capacity. Moreover, antifungal and antibacterial capacities on organic/inorganic materials will be tested. The polysaccharides consolidating and regenerative properties will be analysed over the time by MO, FTIR, SEM-EDS, imaging UV, UV-VIS-NIR, in comparison with conventional treatments.

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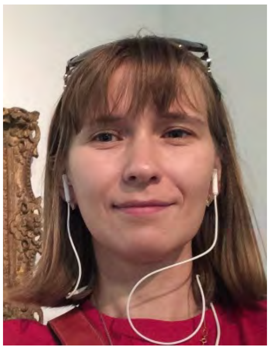

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Molecular oxygen and evolution of the photosynthetic apparatus: the lessons of how to deal with what you produce and what kills you

Keywords: photosystem I, superoxide radical, evolution

Once phototrophs engaged H_2O as the ultimate electron donor for photosynthetic reactions, they encountered the problem of molecular oxygen, which was produced as a byproduct of H_2O photolysis. The O_2 molecule has great potential to interact with the photosynthetic apparatus producing deleterious reactive oxygen species such as singlet oxygen, superoxide radical ($O_2^{\bullet-}$), and hydrogen peroxide. Moreover, the efficient electron flow to O_2 from the components of the photosynthetic chain would decrease the quantum yield of photosynthesis. Here I review recent knowledge about mechanisms of $O_2^{\bullet-}$ formation by the photosynthetic chain, especially within photosystem I, which is inferred to be the major place of this process. Based on a comparison of photosystem I with other I-type reaction centers present in modern anoxygenic phototrophs (*Heliobacteria*, *Chlorobiaceae*, and *Chloracidobacterium thermophilum*), I conclude about possible changes in reaction centers' structure and electron transfer chain that may have been warranted by the need to function in the presence of oxygen.

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Dual role of autophagy in astaxanthin production and cell survival of *Haematococcus lacustris* under salinity

Keywords: autophagy; *Haematococcus*

The microalga *Haematococcus lacustris* (formerly *H. pluvialis*) is able to accumulate high amounts of the carotenoid astaxanthin in the course of adaptation to stresses like salinity. Technologies aimed at production of natural astaxanthin for commercial purposes often involve salinity stress; however, after the switch to stress conditions, *H. lacustris* experiences massive cell death which negatively affects astaxanthin yield. This study addressed the possibility to improve cell survival and astaxanthin yield in *H. lacustris* subjected to salinity via manipulation of the levels of autophagy. The role of autophagy in cell survival was addressed using AZD8055, a known inhibitor of TOR kinase previously shown to accelerate autophagy in several microalgae, while the possible participation of autophagy in astaxanthin synthesis was analysed by transmission electron microscopy. Addition of NaCl to the growth medium induced formation of autophagosomes in *H. lacustris*, while simultaneous addition of AZD8055 up to a final concentration of 0.2 µM further stimulated this process. AZD8055 significantly improved the yield of *H. lacustris* cells after 5 days of exposure to moderate (0.2 % w/v), but not to high (0.8 % w/v) salt stress. Strikingly, this occurred by acceleration of cell growth, and not by acceleration of aplanospore formation. Cytological studies of cells exposed to high (0.8 % w/v) salt stress suggested a role of autophagosomes, lysosomes and Golgi in the process of astaxanthin synthesis from beta-carotene.

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The potential of the phototrophic bacterium *Rhodobacter sphaeroides* in bioremediation of heavy metal ions

Keywords: purple bacteria, biosorption, nickel, cobalt, chromate, bioreduction, Langmuir

Rhodobacter sphaeroides is a purple non-sulphur bacterium well known for its widely investigated photosynthetic reaction centre. Its ability to tolerate environmental stress due to a variety of heavy metal ions has been extensively investigated and assessed.[1,2] The presence of heavy metal ions in the growth medium produced differentiated responses at the level of cellular yield, growth rate, lag phase length and pigment concentration. Moreover, the microorganism showed passive and active mechanisms of metal tolerance, such as biosorption and bioreduction, which encourage the employment of *Rhodobacter sphaeroides* biomass in bioremediation field. Extracellular uptake of nickel(II) and bioreduction of chromium(VI) represent two well-established adaptation mechanisms occurring in this bacterium with high potential for water remediation purposes. Standard and unconventional techniques have been applied to characterize structural and thermodynamic aspects of both processes. Specifically, the results achieved by Langmuir modelling, perfusion induced ATR-FTIR difference spectroscopy and X-ray photoelectron spectroscopy will be presented and discussed.

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Algal biosensors for environmental monitoring

Keywords: algae, nanomaterials, paper-based biosensors, optical/electrochemical biosensors

Algal biosensing is actively contributing to the realization of analytical devices for environmental monitoring with improved performance in term of sensitivity, reproducibility, fast response, and no need for sample pre-treatments [1-4]. In this scenario, we present examples of biosensors designed exploiting the green alga *Chlamydomonas reinhardtii*, in combination with advanced sustainable materials (e.g. paper) and nano-materials (e.g. carbon black), for the detection of diverse target analytes (e.g. photosynthetic herbicides and pathogens). An optical eco-designed paper-based device was developed for optical detection of nanoencapsulated-atrazine in tap water in the picomolar range, where algae were immobilised on a paper substrate soaked with agar thin film [5]. An amperometric biosensor was realised using *C. reinhardtii* algae immobilized on carbon black modified screen-printed electrodes for the amperometric detection of photosynthetic herbicides in river water in the nanomolar range [6]. More recently, a dual opto-electrochemical biosensing platform was realised based on 28 *C. reinhardtii* strains, immobilised on paper-based screen-printed electrodes nanomodified with carbon black, for the detection of atrazine, terbuthylazine, and diuron in tap and surface waters with detection limits in the picomolar ranges [7]. Pathogen detection was also accomplished exploiting *C. reinhardtii* CC125 strain associated to a dual electro-optical transduction prototype ad hoc designed for the algal photosynthetic process. Escherichia coli was exploited as case study pathogen to assess the algae capability to sense their presence in wastewater in a concentration range from 100 to 2000 CFU / 100 mL, with a detection limit of 92 CFU/mL [8]. Moreover, an optical biosensor was designed using peptidomimetics, bioinspired to the D1 protein of the photosystem II of *C. reinhardtii*, functionalised with quantum dots nanoparticles for the detection of atrazine, a case study herbicide widely exploited in agriculture and often found in wastewater, with detection limits in the µg/L concentration range, meeting the requirements of E.U. legislation [9].

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Assessment of ecological state of natural water bodies

Keywords: environmental monitoring, photosynthetic activity, chlorophyll fluorescence

Environmental monitoring is essential for effective management of aquatic ecosystems. Phytoplankton form the basis of life in natural water bodies; its photosynthetic activity provides information about physiological state of primary producers and, hence, about ecological state of the whole aquatic ecosystem. Therefore, efficient monitoring of aquatic ecosystems requires non-invasive, highly sensitive, and real-time techniques to assess photosynthetic parameters of phytoplankton in situ in a wide range of chlorophyll concentrations. Earlier, we designed a fluorometer for automated measurements of high-resolution chlorophyll fluorescence kinetics: light induction (OJIP) and dark decay (Q_A^- reoxidation) kinetics (Antal et al. 2018, Physiol. Plantar.). In order to perform data processing, an original method of spectral multi-exponential approximation was applied. The setup was successfully employed for the continuous monitoring of microalgae in photobioreactor under stress conditions. Our current research is focused on developing more advanced apparatus capable of monitoring phytoplankton activity in their natural environment. New device is intended to measure three types of chlorophyll fluorescence kinetics (light induction of the fast and delayed fluorescence, and fluorescence dark decay curve), as well as phytoplankton absorption spectra. To analyse large arrays of experimental data, artificial neural networks were trained to recognize toxic effects of pollutants on phytoplankton. Further, we intend to develop an automatic system of distributed measuring stations with centralized data collection, data treatment and visualization for the continuous monitoring of large natural water reservoirs.

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