PURPLE4LIFE

PURPLEGAIN (CA21146)
PURPLE4LIFE (CBE-JU 101212806)

WORKSHOP - "BOOSTING THE USE OF PPB AS INNOVATIVE FOOD AND FEED INGREDIENTS: FROM METABOLISM TO HEALTH-PROMOTING EFFECTS"

19 - 20 Mach 2026
University of Mons – Belgium

BOOK OF ABSTRACTS

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Circular Bio-based Europe Joint Undertaking
Bio-based Industries Consortium
Co-funded by the European Union
cost EUROPEAN COOPERATION IN SCIENCE & TECHNOLOGY
PurpleTech Sustainable Innovation in Feed and Food

PURPLE4LIFE

PIONEERING PURPLE PHOTOTROPHIC BACTERIA
FOR SUSTAINABLE FOOD & FEED PRODUCTION

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PURPLEGAIN (CA21146) **PURPLE4LIFE** (CBE-JU 101212806) – Workshop "Boosting the use of PPB as innovative food and feed ingredients: From metabolism to health-promoting effects"

Design and layout: Sociedade Portuguesa de Inovação | Grupo Ciberimaginario (URJC)

INDEX

Day 2

Abstract selected for poster

WELCOME MESSAGE

Welcome to the workshop “Boosting the Use of PPB as Innovative Food and Feed Ingredients: From Metabolism to Health Promoting Effects”. This event represents a joined initiative from the PurpleGain COST Action and the Purple4Life CBE-JU project, both dedicated to the development of phototrophic purple bacteria-based processes. We hope to make it a significant moment for the international community studying these microorganisms and their potential contribution to sustainable food and feed systems. This event brings together researchers, technologists, industry actors and policy stakeholders interested in advancing knowledge about PPB metabolism, biotechnology and health related applications.

The workshop constitutes the tenth scientific meeting organized within the framework of the COST Action PurpleGain (CA21146) and at the same time the first workshop linked to the European research project Purple4Life (CBE-JU 101212806). Both initiatives aim to strengthen collaboration across disciplines including microbiology, biotechnology, food science, nutrition and environmental sustainability research.

Purple phototrophic bacteria are increasingly recognised as promising microbial resources capable of converting diverse organic substrates including residual biomass and waste streams into valuable products such as proteins, pigments, vitamins, antioxidants and many other bioactive compounds with potential applications in food, feed and health related innovations.

This Book of Abstracts gathers the scientific contributions presented during the workshop and reflects the diversity of current research exploring PPB metabolism, bioprocess optimisation and functional ingredient development. The workshop is organised by partners of the Purple4Life project together with the COST Action PurpleGain network. Activities of PurpleGain are funded by COST European Cooperation in Science and Technology while Purple4Life receives support from European.

PURPLE4LIFE 2026

INVITED SPEAKERS

PURPLEGAIN (CA21146)
PURPLE4LIFE (101212806)

WORKSHOP

"BOOSTING THE USE OF PPB AS INNOVATIVE FOOD AND FEED INGREDIENTS: FROM METABOLISM TO HEALTH-PROMOTING EFFECTS"

INVITED SPEAKER

LIGHT CAPTURE IN PPB: FUNDAMENTALS AND PERSPECTIVES

Andrew Hitchcock

Royal Society Research Fellow/ Lecturer of School of Biosciences. University of Sheffield, UK

INVITED SPEAKER

PROTEIN FROM VARIOUS WASTEWATERS VIA PHOTOSYNTHETIC BACTERIA

Mari Eskola

Dr, Senior Regulatory Affairs Expert at Medfiles, Finland

INVITED SPEAKER

REGULATORY PATH TO THE FEED ADDITIVE MARKET

Marta Ponghellini

Deputy Head of Unit in the Animal Nutrition-Veterinary Medicines division at the DG SANTE

INVITED SPEAKER

FROM AUTHORISATION TO OFFICIAL CONTROL: WHICH ANALYTICAL METHODS REALLY MATTER FOR FEED ADDITIVES?

Christoph von Holst

Active senior at the European Commission, Joint Research Centre, Geel, Belgium

INVITED SPEAKER

INNOVATIVE INGREDIENT FOR IMPROVING FEED IN AQUACULTURE

Katerina Kousoulaki

Senior researcher in nutrition at Nofim, Norway

INVITED SPEAKER

COQ10 AND HUMAN HEALTH

Plácido Navas

Senior researcher. Andalusian Centre for Developmental Biology (CABD) UPO

PURPLE4LIFE 2026

SPEAKER ABSTRACT

PURPLEGAIN (CA21146)
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WORKSHOP

"BOOSTING THE USE OF PPB AS INNOVATIVE FOOD AND FEED INGREDIENTS: FROM METABOLISM TO HEALTH-PROMOTING EFFECTS"

Speaker Abstract

From Authorisation to Official Control: Which Analytical Methods really matter for Feed Additives?

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The European Union Reference Laboratory for Feed Additives (EURL-FA) plays an important role in the authorisation of feed additives in the European Union. Its activities are defined in the framework of Regulation (EC) No 1831/2003 on additives for use in animal nutrition, which established a harmonised authorisation procedure for feed additives and requires the scientific assessment of analytical methods used for their official control.

The primary task of the EURL is to evaluate and verify the analytical methods proposed by applicants for the determination of feed additives in feed, premixtures, and where relevant in animal-derived products. In this task the EURL is supported by network of National Reference Laboratories. These methods are submitted as part of the authorisation dossier. The EURL-FA examines whether the methods are suitable for regulatory purposes, including their applicability, reliability, performance characteristics, and compliance with internationally accepted standards.

The EURL-FA also manages a repository of feed additive samples provided by the applicant.

Beyond the authorisation procedure, the EURL contributes to method development, validation, and standardisation, and promotes best practices for the analysis of feed additives. Through its scientific and technical activities, the laboratory ensures that reliable analytical tools are available to support regulatory decisions and effective monitoring of feed additives in the European Union.

List of key legislation regarding the authorisation of feed additives, available from the following website. Please check also the consolidated versions of the regulations.

https://eur-lex.europa.eu

- *Authorisation of feed additives*: European Union. 2003. Regulation (EC) No. 1831/2003.
- *Preparation of dossiers*: European Union. 2008. Commission Regulation (EC) No. 429/2008
- *Task and operation of the EURL*: Commission Regulation (EC) No. 378/2005
- *Analytical methods in the field of feed*: Commission Implementing Regulation (EU) 2024/771 amending Regulation (EC) No 152/2009

EURL guidelines: https://joint-research-centre.ec.europa.eu/eurl-fa-eurl-feed-additives/eurl-fa-authorisation/eurl-fa-guidance-applicants_en

Publications

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- von Holst C., Robouch P., Bellorini S., Gonzálezde la Huebra M.S. & Ezerskis Z. A review of the work of the EU Reference Laboratory supporting the authorisation process of feed additives in the EU. Food Additives & Contaminants: Part A, (2016), 33:1, 66-77; *available from the speaker*
- Vincent U., Serano F. and von Holst C. Development and validation of a multi-analyte method for the regulatory control of carotenoids used as feed additives in fish and poultry feed. Food Additives & Contaminants: Part A, (2017) Vol. 34, 8, 1285–1297; https://doi.org/10.1080/19440049.2017.1315651 (open access)
- Vincent U., Serano F. and von Holst C. Validation of a multi-analyte HPLC method for the determination of carotenoids used as feed additives in fish and poultry feed: results of an interlaboratory study. Food Additives & Contaminants. 2021: 38, 3, 396–408 https://doi.org/10.1080/19440049.2020.1869325 (open access)

PURPLE4LIFE 2026

SELECTED ABSTRACTS FOR ORAL PRESENTATIONS

DAY 1

PURPLEGAIN (CA21146)
PURPLE4LIFE (101212806)

WORKSHOP

"BOOSTING THE USE OF PPB AS INNOVATIVE FOOD AND FEED INGREDIENTS: FROM METABOLISM TO HEALTH-PROMOTING EFFECTS"

Day 1 – Session I

Environmental Stress as a Driver of Pigment Modulation in Rhodobacter capsulatus

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The transition toward a circular bioeconomy requires microbial platforms capable of converting waste-derived substrates into valuable biomolecules while operating under environmentally relevant conditions. Purple phototrophic bacteria (PPB), such as *Rhodobacter capsulatus*, are promising candidates due to their metabolic versatility and ability to simultaneously produce microbial protein and high-value carotenoids [1]. These pigments exhibit strong antioxidant properties and are of interest for nutraceutical, cosmetic, and feed applications, while also playing a key role in microbial photoprotection and oxidative stress mitigation [2].

This work explores a biological solution based on the controlled modulation of carotenoid biosynthesis in *R. capsulatus* through environmental stressors, specifically oxygen availability and UV-C exposure. By adjusting cultivation conditions, pigment production and composition can be modified, offering a flexible and scalable process strategy. Pigments were extracted using organic solvents and characterized by UV–Vis spectrophotometry and HPLC-DAD, allowing the identification of stress-induced shifts in carotenoid profiles.

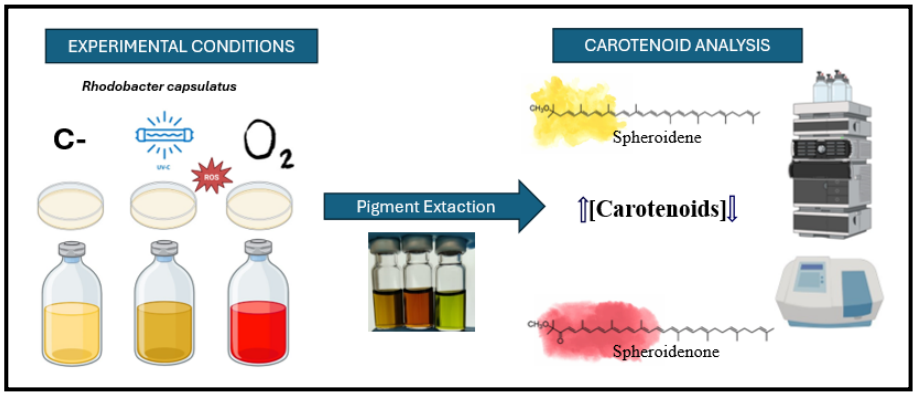

Figure 1. Graphical abstract

Exposure to UV-C radiation markedly reduced the survival of *Rhodobacter capsulatus* colonies, reflecting the DNA-damaging effects of UV-C (Figure 2). Surviving populations showed an overall increase in total carotenoids, indicating that enhanced pigment biosynthesis contributes to coping with UV-induced oxidative stress (Figure 3). Oxygen availability strongly influenced culture appearance and pigment composition. Aerobic cultures appeared red, while anaerobic cultures remained yellowish, a difference also reflected in pigments extracted with organic solvents. Spectrophotometric and HPLC analyses confirmed that oxygen

promoted the CrtA-mediated conversion of spheroidene into spheroidenone, enhancing carotenoid photoprotection (Figure 4) [3]. The resulting color shift serves as a visible marker of this metabolic adaptation, highlighting oxygen as a key regulator of pigment biosynthesis *in R. capsulatus*.

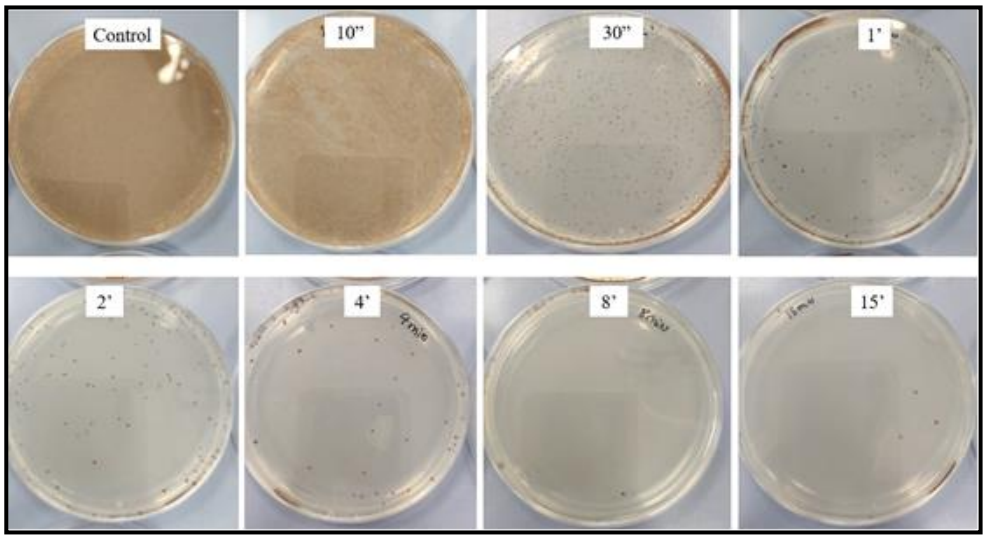

Figure 2. Effect of UV-C light stress on *Rhodobacter capsulatus* colonies. Cultures were exposed to different doses of UV-C, showing a gradual decrease in colony survival with increasing exposure times

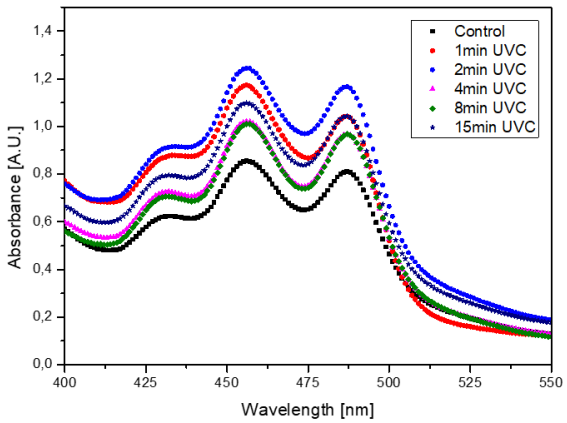

Figure 3. UV–Vis absorption spectra of carotenoid extracts from cultures exposed to different UV-C exposure times compared with the non-irradiated control

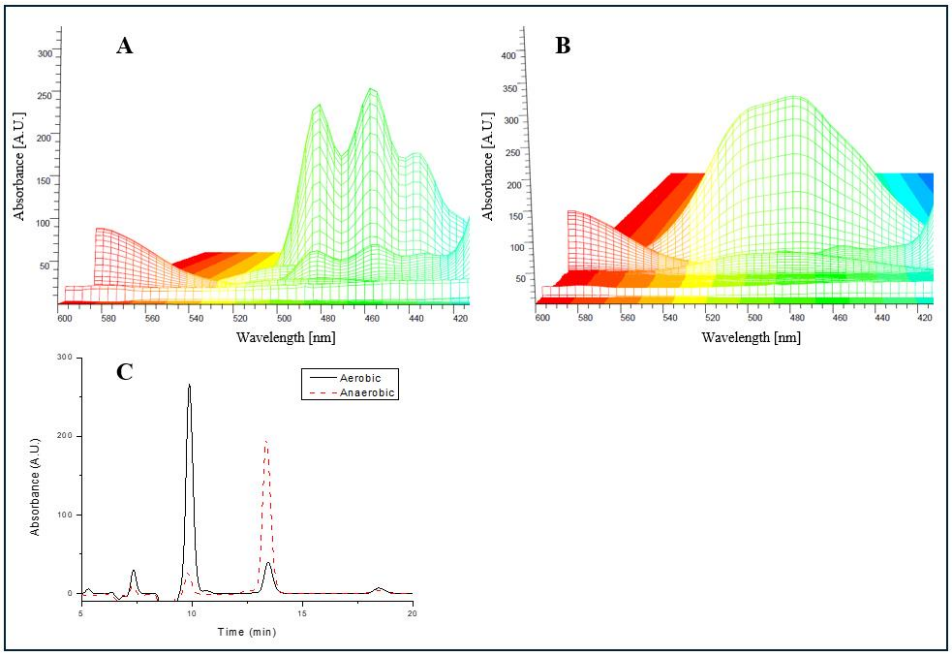

Figure 4. Spectral characterization and pigment analysis of *R. capsulatus* under different oxygen conditions. Absorbance spectra recorded under anaerobic conditions, showing a yellow phenotype dominated by spheroidene (A). Absorbance spectra recorded under aerobic conditions, showing a red phenotype dominated by spheroidenone (B). HPLC chromatograms showing clear separation of carotenoids from both extracts. Peaks corresponding to spheroidene (anaerobic) and spheroidenone (aerobic) are distinctly resolved (C)

These findings demonstrate that *Rhodobacter capsulatus* actively adjusts carotenoid metabolism in response to environmental stress, enhancing photoprotection and shifting pigment composition. Such adaptive responses not only highlight opportunities to optimize cultivation for high-value carotenoid production but also reinforce the sustainability potential of coupling waste valorization with bio-based product generation. Participation in this workshop will provide insights into scaling strategies, regulatory considerations, and market positioning, while fostering connections with stakeholders in biorefinery development and sustainable biotechnology.

Acknowledgements:

This work was financially supported by the CBE-JU under the EU's Horizon Europe research and innovation programme (Purple4Life, Grant Agreement No. 101212806) and by the Comunidad de Madrid through the Technology Networks call, funding BIVALIA-CM project (TEC-2024/BIO-177).

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Bioelectrochemical production of food-grade value-added compounds by *Rhodopseudomonas* during biogas upgrading

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One of the current challenges faced by society is sustaining the growing food demand driven by the continuous increase in the global population, which is projected to increase by 50% - 98% by 2050 [1]. Addressing this challenge requires the development of food systems that operate in a more sustainable manner. Current estimates up to 34% of global greenhouse gas emissions to the agrifood sector, which puts pressure on natural resources. In this context, food microbiology emerges to promote sustainability throughout the entire food production process [2].

This study develops a bioelectrochemical photobiorefinery concept based on purple phototrophic bacteria (PPB) from the genus *Rhodopseudomonas* to upgrade biogas into biomethane while co-producing food-grade value-added compounds. PPB can fix CO_2 under photoheterotrophic, photoautotrophic, or photoelectrotrophic conditions, including direct electron uptake from electrodes [3]. As a result, this biomass can be enriched in compounds such as CoQ10 an antioxidant with benefits for several diseases such as cardiovascular, cancer, and neurodegenerative disorders [4]. In this way, the aim is not only to upgrade biogas by converting it into biomethane, but also to obtain value-added compounds in a more sustainable way.

The objective of this work was to select the *Rhodopseudomonas* strain with the highest capacity for inorganic CO_2 fixation and CoQ10 accumulation, targeting its application in a photo-bioelectrochemical system. Four strains were evaluated under different metabolic regimes, including photoheterotrophy, photoheterotrophy with CO_2 fixation using butyrate as a reduced carbon source, and photoelectroautotrophy in a single-chamber photo-microbial electrolysis cell with a poised cathode (Figure 1).

The results of the PPB growth experiments revealed two phases (Figure 2). Up to day 7, all cultures showed limited growth, with TIE-1 growth on butyrate exhibiting the lowest OD. After day 7, TIE-1 on butyrate displayed the highest growth rate, whereas the mixed culture and TIE-1 on acetate stabilized, reaching similar final OD values. Organic matter consumption followed the same pattern. These differences are attributed to the distinct redox states of acetate and butyrate. CO_2 fixation occurred from the beginning in the mixed culture, while TIE-1 showed an acclimation phase before sustained fixation.

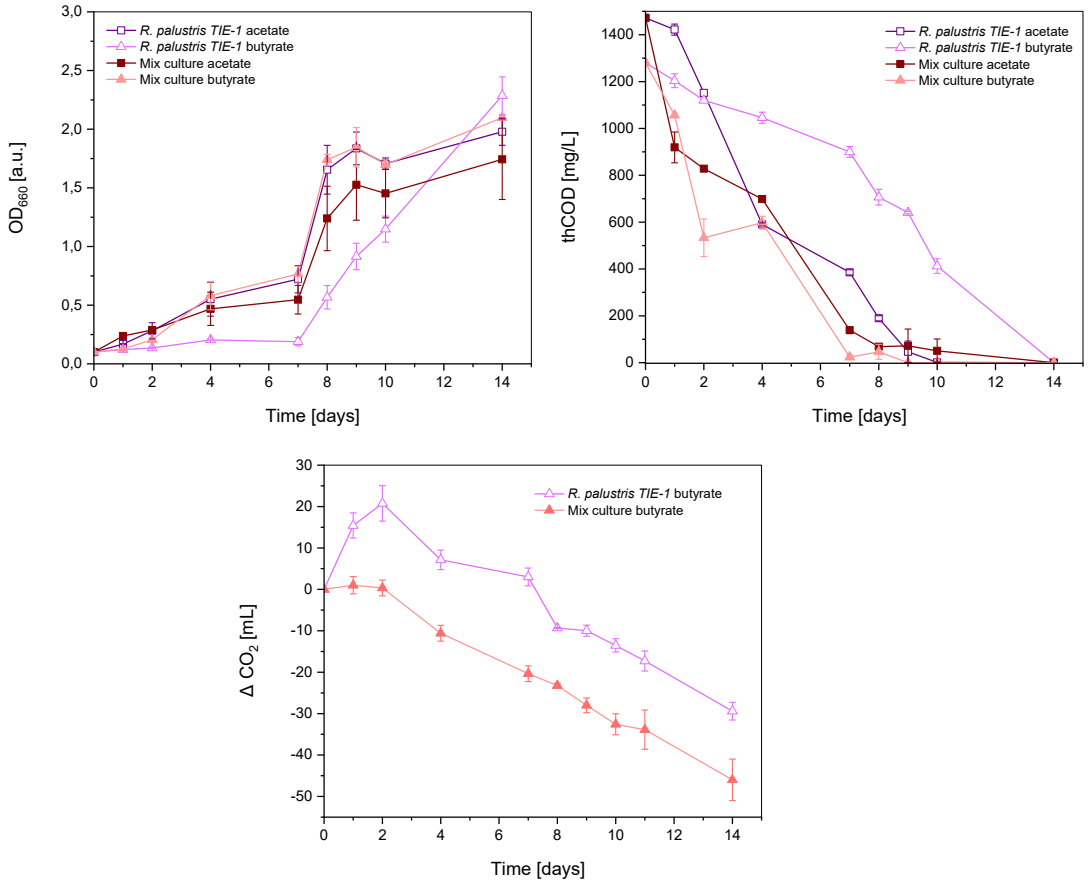

Figure 1. Biomass growth of PPB based on optical density at 660 nm (A), and theoretical Chemical Oxygen Demand consumption (B). Cumulative CO_2 fixation by R. palustris TIE-1 and by themixed culture (C). The error bars represent the standard error (n=2).

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Impact of salinity on pigments and antioxidant properties of purple bacteria

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Background. Purple bacteria (PB) are emerging as sustainable sources of high-value nutraceuticals. They produce protein-rich biomass enriched with carotenoids, health-promoting compounds such as coenzyme Q10 (CoQ10), and other antioxidants for food and feed applications [1]. While salinity has been explored to enhance carotenoid synthesis, its effect on overall antioxidant capacity remains largely unexplored, but is important for PB-based bioprocesses [2],[3].

Aim. This study evaluated how salinity influences the content and productivity of pigments and antioxidants for three halotolerant purple non-sulphur bacteria.

Methods. *Rhodobacter (Rb.) capsulatus* (freshwater), *Cereibacter (Cb.) sphaeroides*, and *Afifella (Af.) marina* (salt-tolerant) were cultivated in batch experiments across six salinities (1, 11.5, 23, 35, 50, 70 g L^{-1} NaCl). We quantified total antioxidant capacity (phosphomolybdenum assay), carotenoids (spectrophotometry), and CoQ10 (C30-HPLC).

Results. Salinity strongly affected all targeted compounds and growth rates, but responses were species-specific. Antioxidant capacity and carotenoid content generally declined with increasing salinity, with *Cb. sphaeroides* showing the highest values at low salinity (≈29 mg AAE g^{-1} DW) but reduced performance beyond 35 g L^{-1}. *Rb. capsulatus* was the most salt-sensitive, with steep pigment and antioxidant losses that matched its freshwater origin. In contrast, *Af. marina* displayed the highest resilience: although absolute values remained lower, its antioxidant and carotenoid levels decreased more gradually, and it had the highest carotenoid productivity ≥50 g L^{-1} NaCl. CoQ10 trends diverged from carotenoids. *Cb. sphaeroides* maintained the highest CoQ10 content across most conditions (4.6–6.1 mg g^{-1} DW), while *Af. marina* showed a steady increase with salinity and *Rb. capsulatus* displayed moderate increases. The opposite salinity responses of carotenoids and CoQ10 in some species suggest metabolic rerouting of the shared precursor geranylgeranyl diphosphate under osmotic stress [4].

Conclusion and outlook. Three halotolerant PB maintained pigment and antioxidant profiles up to ~23–35 g L^{-1} NaCl, defining a practical salinity range for bioprocesses operating under low to moderate salinities. These salinity-dependent responses offer opportunities for several biotechnological applications aimed at enhancing natural pigment or antioxidant production. Depending on the species and target compound, medium salinity can be selected to steer metabolite yields and productivities toward the desired application.

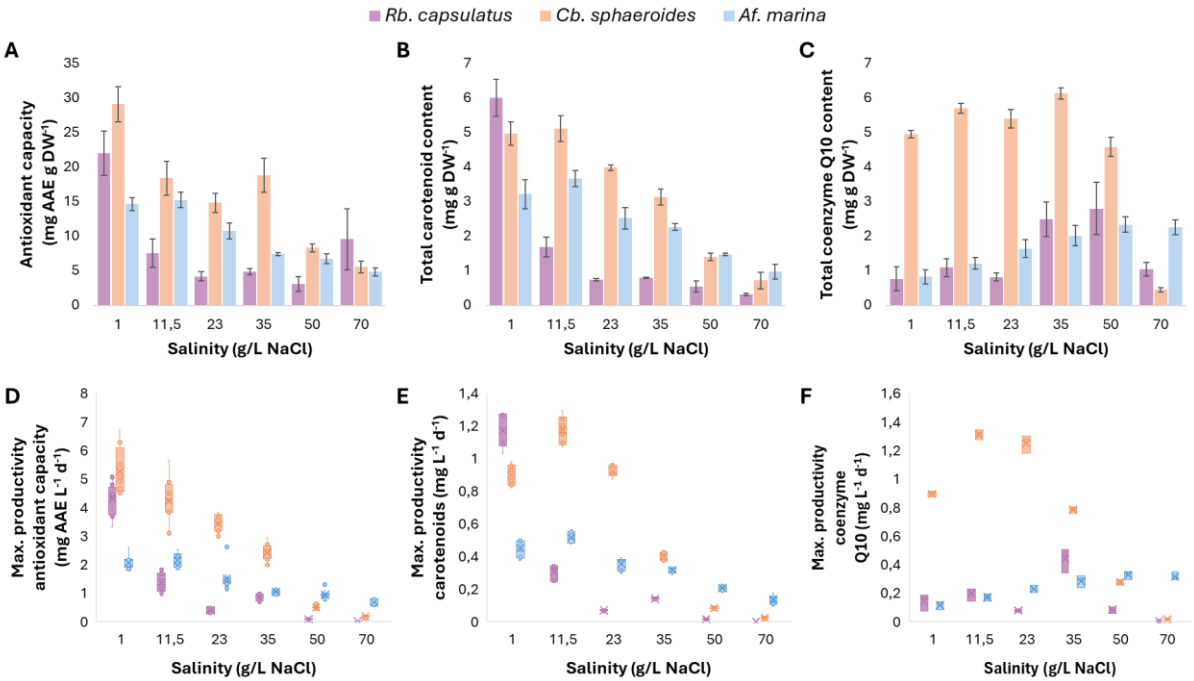

Figure 1. Effect of NaCl concentration on the content (A-C) and productivity (D-F) of antioxidant capacity (n=12; A & D), carotenoids (n=6; B & E) and coenzyme Q10 (n=3; C & F) in three purple non-sulfur bacteria. Bars represent mean values ± standard deviation for *Rhodobacter capsulatus* (pink), *Cereibacter sphaeroides* (orange) and *Afifella marina* (blue) grown under increasing NaCl concentrations (1, 11.5, 23, 35, 50, 70 g L^{-1}). Boxplots represent the median value, while the box borders represent quartiles Q1 and Q3. Outliers in the dataset are represented with a dot.

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Effective light management as a lever to improve productivity in purple phototrophic bacteria

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Purple phototrophic bacteria (PPB) are emerging microbial platforms for the sustainable, light-driven production of high-value metabolites such as carotenoids and other redox-active compounds [1]. In phototrophic bioprocesses, light acts both as the primary energy source and as a key operational parameter controlling biomass productivity and metabolite yields. However, increasing irradiance to enhance production often leads to heterogeneous light distribution, photoinhibition, and oxidative stress, particularly in dense cultures typical of industrial systems. As a consequence, optimization strategies based solely on incident irradiance may poorly predict microbial performance and product formation.

In this study, six PPB species were cultured under incident irradiances ranging from 0 to 230 µmol photons m^{-2} s^{-1}. Growth dynamics were monitored and fitted using the Gompertz model to estimate the maximum specific growth rate (μ). Biomass-dependent light attenuation within the cultures was described using the Lambert–Beer law [2]. Attenuation parameters were determined from culture-specific measurements and from flask geometry. These parameters were combined to calculate the average effective irradiance experienced by the cells (I_{av}). The relationship between μ and I_{av} was analyzed using light-response models without photoinhibition (Monod and Grima) and with photoinhibition (Steele and Haldane) [3]. At the end of the cultures, carotenoids were extracted from biomass and quantified spectrophotometrically relative to dry cell weight.

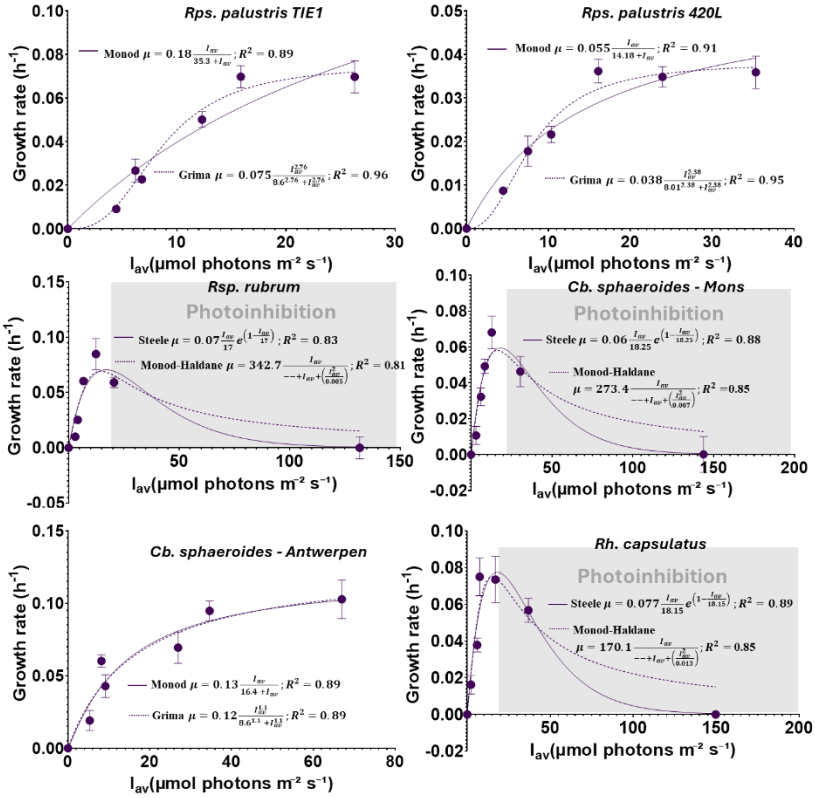

Figure 1. Light-dependent growth and photoinhibition of PPB

Growth rates increased with I_{av} under light-limiting conditions and reached saturation at intermediate I_{av} in all strains. At high I_{av}, photoinhibition was observed in *Rsp. rubrum*, *Cb. Sphaeroides*-Mons, and *Rh. Capsulatus* (Fig. 1). Carotenoid accumulation generally peaked at low to intermediate irradiance (17–30 µmol photons m^{-2} s^{-1}) in most strains and declined at higher irradiance, while *Cb. sphaeroides*-Antwerpen sustained elevated carotenoid levels under high light, reaching ~1.8 mg g^{-1} at 230 µmol photons m^{-2} s^{-1}.

Overall, this work demonstrates that light exerts species-specific effects on growth and metabolite accumulation in PPB. The application of different light-response models reveals interspecific variability and represents a step toward optimising PPB productivity. Future studies will extend this approach to different light qualities and additional metabolites, such as coenzyme Q10.

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Day 1 – Session II

Safety Evaluation of Purple Phototrophic Bacteria Biomass Using In Vitro and In Silico Methods

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Novel food safety assessment entails a rigorous scientific evaluation by authorities such as EFSA, addressing toxicology, nutritional adequacy, allergenicity, and production processes to ensure consumer safety prior to market authorization. These findings form the basis for risk management, which involves selecting appropriate policy measures (e.g., labeling requirements or usage restrictions) while considering socio-economic implications and the effectiveness of strategies for consumer protection.

The safety assessment of Purple Phototrophic Bacteria (PPB) biomass as novel food is a key prerequisite for its potential application biotechnological sectors for food and feed. The proposed approach addresses a preliminary safety evaluation strategy based on in vitro and in silico methodologies, in accordance with European Food Safety Authority (EFSA) guidance and the principles of Next Generation Risk Assessment (NGRA). The approach focuses on two critical aspects of safety: cytotoxicity and protein allergenicity.

In vitro cytotoxicity assessments are conducted using established human cell culture models representative of relevant exposure routes and biological systems. Time- and dose–response studies are performed to assess potential adverse cellular effects of PPB biomass and its main components, employing standardized viability assays to establish cytotoxic thresholds and perform hazard characterization in support of risk assessment. Where relevant, functional cellular responses are further explored to distinguish between adverse and biologically active effects.

Protein allergenicity is evaluated using a weight-of-evidence framework that combines *in silico* and *in vitro* analyses, as recommended by EFSA for novel proteins[1]. *In silico* bioinformatics tools are employed to assess sequence similarity between PPB proteins and known allergens, enabling the identification of potential IgE cross-reactivity based on internationally accepted criteria. Complementary in vitro assays assess protein stability under simulated gastrointestinal conditions, providing insight into characteristics associated with allergenic potential. All analyses are supported by advanced proteomic and bioinformatic platforms to ensure robust protein identification and characterization.

This work will pave the way for regulatory compliance and the safe use of PPB as a novel food and feed ingredient, facilitating its transition from basic research to health-oriented applications in the food and feed sectors.

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Checking the environmental relevance of bacterial biomass as a food product – LCA in the PROTEBoost project

Olivier Talon, Guillaume Bayon-Vicente, Baptiste Leroy

The PROTEBoost project aims at developing protein-rich food products based on purple bacteria, using molasses as a substrate for *Rhodospirillum rubrum* biomass production.

In PROTEBoost, a Life Cycle Assessment (LCA) study is performed along the project with a double objective: identifying potential environmental hotspots in the production process to guide optimisation efforts in an eco-design perspective and positioning the products versus market available products (meat or vegetable-based products).

In this work we describe the life cycle inventory of the whole production process, encompassing culture medium formulation, bacterial growth process in photobioreactors, downstream harvesting and concentration processes, and integration of the biomass as an ingredient of a food product, here a burger (figure 1). The inventory was modelled with Simapro 10.2 software, using Ecoinvent 3.11 and Agribalyse 3.2 as background databases. Environmental indicators were calculated with the Environmental Footprint 3.1 calculation method.

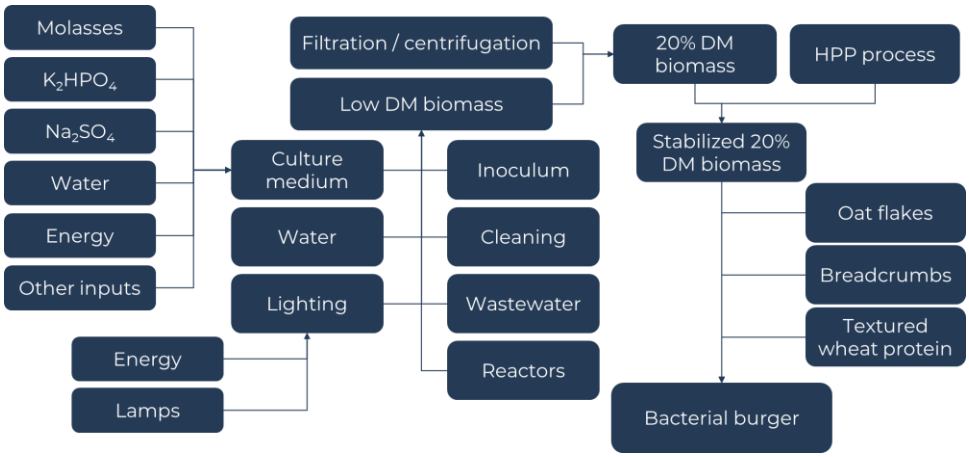

Figure 1. Bacterial burger production system

The contribution analysis of the results reveals that the biomass is the main contributor to the calculated impacts of the food burger (figure 2), this contribution being mostly dominated by the energy for lighting (figure 3).

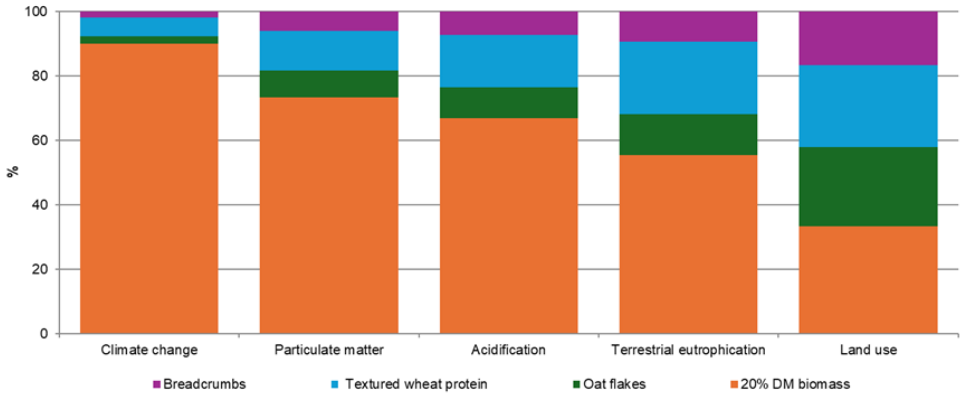

Figure 2. Environmental impacts of a burger – Contribution analysis – Selected indicators

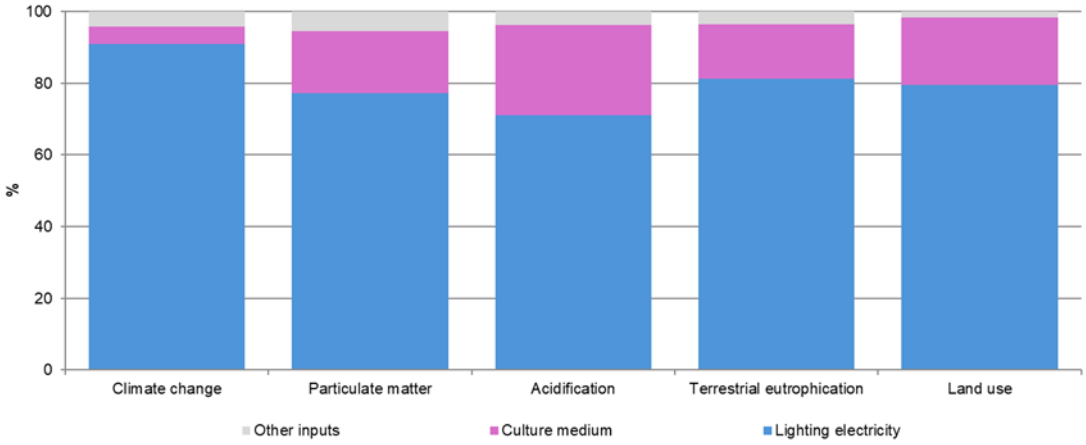

Figure 3. Impacts of biomass production – Contribution analysis – Selected indicators

The comparison with meat and vegetable-based benchmark burgers shows that purple bacteria biomass-based food products can reach environmental competitivity when compared to meat or even vegetable alternatives (figure 4). A key parameter for optimised impacts is the efficiency of the lighting of the photobioreactors.

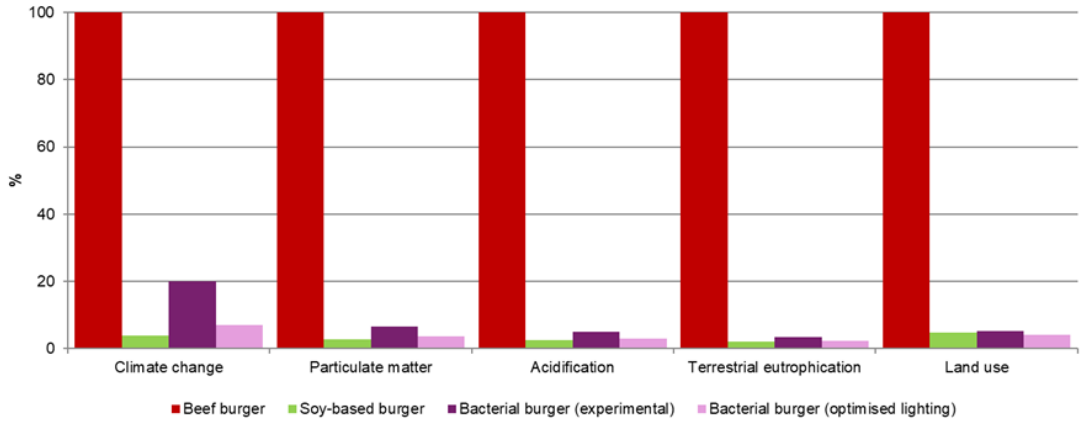

Figure 4. Compared impacts of biomass burgers and benchmarks – Impacts for 100 g protein – Selected indicators

This work aims to share insights on the potential sustainability of purple bacteria as innovative food ingredients and engage with experts to discuss viable process optimisations.

The research leading to these results has been funded by the Public Service of Wallonia (Economy, Employment and Research), under the FoodWal agreement n°2210182 from the Win4Excellence project of the Wallonia Recovery Plan

Directed evolution for improving the upcycling of glucose-rich by-products using phototrophic purple bacteria

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The circular economy has emerged as a key strategy to address both economic and environmental challenges, particularly through the valorisation of organic-rich industrial liquid by-products such as wastewater, whey, or molasses. Purple non-sulphur bacteria (PNSB) are promising biocatalysts for such circular bioprocesses due to their high metabolic versatility. However, a major limitation remains: *Rhodospirillum rubrum*, one of the most studied PNSB, has long been considered unable to assimilate glucose[1,2], one of the predominant carbon source in many agri-food effluents. The mechanisms underlying this metabolic limitation have remained unclear, constraining the industrial use of *Rs. rubrum* for carbohydrate-rich waste upcycling. This study addresses the key challenge of enabling glucose assimilation in *Rs. rubrum* to broaden its applicability in circular biotechnologies. Using long-term acclimation under phototrophic and anaerobic conditions, we developed a novel glucose-assimilating strain of *Rs. rubrum*, named the MG strain. This acclimated strain exhibited a glucose assimilation rate (Figure 1A) more than fivefold higher than the wild-type strain (Figure 1B). Genomic and proteomic analyses revealed that mutations within a gene cluster involved in xylitol assimilation were associated with the acquired glucose assimilation capacity. This finding was further supported by the observation that wild-type *Rs. rubrum* was able to assimilate glucose in the presence of xylitol. Importantly, the robustness and scalability of this metabolic adaptation were demonstrated by successful transfer from laboratory-scale synthetic media to photobioreactor operation using molasses as a complex, industrially relevant carbon source. Overall, this work provides both fundamental insights into carbohydrate photo-metabolism in PNSB and a practical solution for expanding the use of *Rs. rubrum* in agri-food waste upcycling. The developed approach enhances process efficiency, supports sustainable resource recovery, and strengthens the market potential of PNSB-based circular bioprocesses.

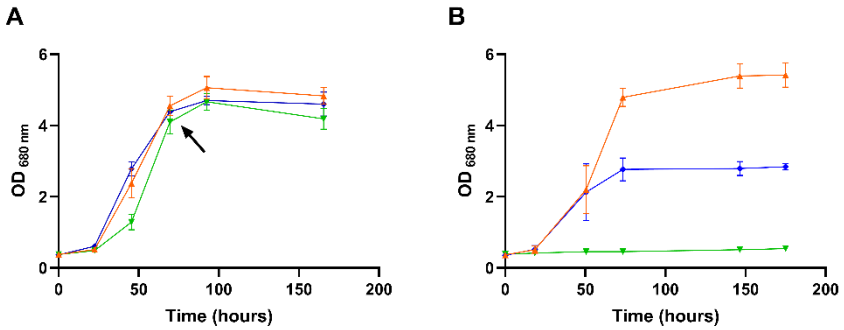

Figure 1. Monitoring of growth of the MG strain **(A)** or the *Rs. rubrum* WT strain **(B)** in medium containing fructose (orange line), glucose (green line) or a mix of fructose and glucose (blue line) as carbon sources. $n = 3$. Results are represented as the mean ± SD

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PURPLE4LIFE 2026

SELECTED ABSTRACTS FOR POSTER PITCHES

DAY 1

PURPLEGAIN (CA21146)
PURPLE4LIFE (101212806)

WORKSHOP

"BOOSTING THE USE OF PPB AS INNOVATIVE FOOD AND FEED INGREDIENTS: FROM METABOLISM TO HEALTH-PROMOTING EFFECTS"

Redefining Molasses for Rhodospirillum rubrum Biomass Production: Metabolic Optimization and Techno-Economic Analysis

Guillaume Bayon-Vicente, Baptiste Leroy

Laboratory of Proteomics and Microbiology, Research Institute for Biosciences
University of Mons, Mons, Belgium.

Description of technology and solutions

The valorisation of sugar beet molasses in microbial bioprocesses has traditionally focused on "downcycling" for photofermentative biohydrogen production. This research shifts the focus toward an "upcycling" strategy, utilizing molasses as a primary substrate for *Rhodospirillum rubrum* biomass production, targeting the food and feed sectors. Using a glucose-acclimated strain (*Rs. rubrum* MG), we optimized macro-nutrient supplementation and culture conditions within laboratory flasks and low-cost bag photobioreactors (PBRs) to ensure industrial scalability.

Technical results and metabolic insights

Findings indicate that supplementation with sulphate and phosphate is essential for enhancing productivity with phosphate acting as a key driver of carbon assimilation and protein accumulation. Sequential batch experiments revealed a strong metabolic coupling between medium acidification through volatile fatty acid production, nutrient availability, and the activation of fermentative pathways (Figure 1). Under optimized conditions, the harvested biomass achieved a protein content exceeding 55% of the dry weight (Figure 2), with preliminary insights on amino acid profile showing the impact of medium optimization.

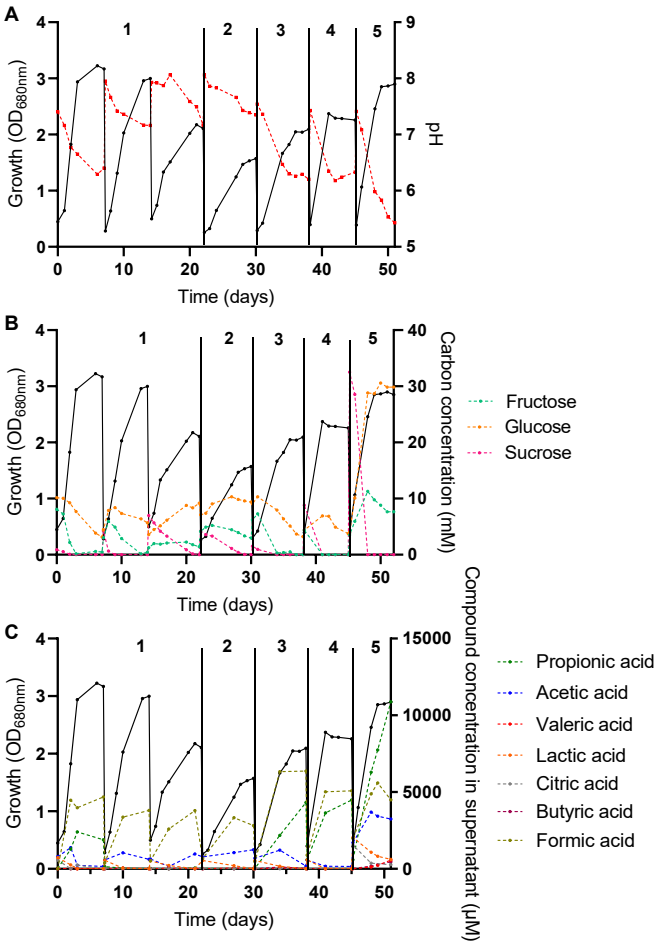

Figure 1. Growth (solid black line), pH (A), sugar concentrations (B), and organic acid production (C) during sequential batch cultivation. In (B), sucrose, glucose, and fructose are shown as red, green, and orange dotted lines, respectively. In (C), formic, acetic, propionic, butyric, valeric, and lactic acids are represented by dark yellow, purple, green, blue, red, and orange dotted lines, respectively. Numbers above the graphs indicate culture phases: (1) initial medium, (2) +Na_2SO_4, (3) +phosphate buffer, (4) +Na_2SO_4 and phosphate, and (5) +Na_2SO_4, phosphate, and 20 g/L molasses

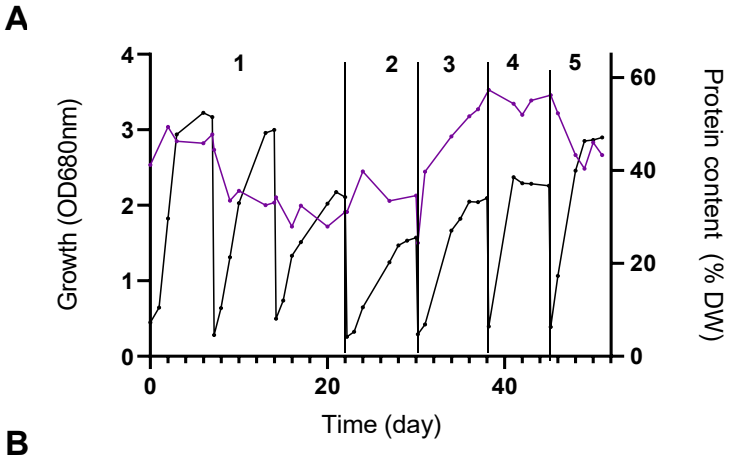

Figure 2. Protein content of Rhodospirillum rubrum MG following during medium composition optimization. Bacterial growth is represented by a solid black line, while protein content is shown as a solid purple line.

Techno-Economic Analysis

A rigorous techno-economic analysis (TEA), based on experimental and literature, involving more than 250 production scenarios and five archetypal cost clusters was conducted (Figure 3). Illumination energy and manpower were identified as the dominant overall expenditure vectors accounting for between 60 to 97% of the total production cost. Achieving a viability threshold of <50€/kg, necessary for high-value markets like nutraceuticals or feed additives, was possible in only one-third of the scenarios. This requires optimized illumination (less than 7 W/L of culture), high productivity (>1.5 g/L.d), and a moderate automation with one operator handling at least 15,000L of culture. Reaching the general food market (<10€/kg) remains extremely challenging, requiring the simultaneous minimization of all cost factors. The study highlights a strategic risk: without significant investments in automation and energy efficiency to mitigate higher European labour and electricity costs, production may shift to regions with superior structural competitiveness.

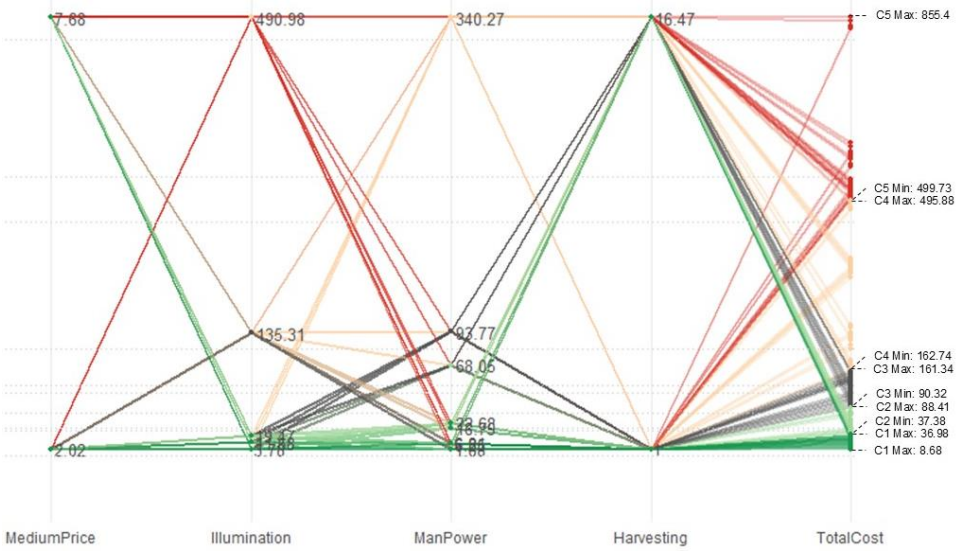

Figure 3. Multifactorial analysis of the biomass production cost

Expectations from the workshop

This work aims to share metabolic insights and engage with experts to bridge the gap between process optimization and industrial economic viability scenarios for purple bacteria as innovative food ingredients.

Electro-stimulation of *Rhodopseudomonas palustris* 42OL for biosynthesis of High-Value Bio-product: Implications for food systems and Sustainability

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*Department of Agriculture, Food, Environment and Forestry (DAGRI), University of Florence, Piazzale delle Cascine,18 and Via Maragliano 77, Florence, Italy

Abstract

Rhodopseudomonas palustris 42OL produces Polyhydroxybutyrate, a versatile biopolymer used as a nutrient-dense protein and prebiotic. To support a circular bio-economy, efficient recovery platforms are essential. This study explores a microbial electrolysis framework to enhance the conversion of carbon sources into these biogenic polyesters and high-value biomass.

This study evaluates the effect of specific cathode potentials (-450 mV and -600 mV) on enhancing polyhydroxybutyrate biosynthesis by *Rhodopseudomonas palustris* 42OL in Microbial Electrolysis Cells. Polyhydroxybutyrate production was assessed using two complementary approaches: direct quantification via High-Performance Liquid Chromatography following hot acid digestion, and indirect cellular PHB analysis using flow cytometry with an optimized protocol. Other parameters such as biomass production, carbon source assimilation, and hydrogen production, were followed during the study.

Our results suggest that electro-stimulation with different applied potentials significantly enhances the yield of these functional molecules; preliminary results showed improved Polyhydroxybutyrate content between the electrically enhanced bacteria and the control in photofermentation. Moreover, it was shown that the potential value could enhance the final yield of Polyhydroxybutyrate production. Furthermore, observing the obtained results using the flow cytometer, we noticed three sub-populations in the same MEC, with different Polyhydroxybutyrate content, which may indicate a metabolic diversity and different behavior adopted by *R. palustris* 42OL in the synthesis of Polyhydroxybutyrate. The results also demonstrated significant *R. palustris* 42OL biomass accumulation during electrochemical induction within the Microbial Electrolysis Cell. These results highlight the significant potential of utilizing purple non-sulfur bacteria within electrochemical systems to maximize the recovery of high-value metabolites and biomass.

Keywords: *Rhodopseudomonas palustris 42OL, bio-electrochemical system, polyhydroxybutyrate, biosynthesis, applied potential*

Acknowledgments:

This work was supported by Ministero italiano dell'Università e della Ricerca during the research program PRIN 2022 PNRR "Valorization of cheese whey by hydrogen production in bio-electrochemical systems catalyzed with purple bacteria (WHISPER)" and by Ministero dell'ambiente e della sicurezza energetica (MASE), Italy, Project SPIGA "Sviluppo di una piattaforma di Produzione di Idrogeno Green mediante sistemi innovativi, UGOV RSH2A_000025_SPIGA_PNRRM2.C2.I3.5".

Purple Phototrophic Bacteria (PPB) production from fermented wine lees: an insight into polyhydroxyalkanoates and pigments synthesis

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This research focuses on using a circular economy model that uses winery lees as raw material to produce phototrophic purple bacteria (PPB) as potential aqua-feeds ingredients, utilizing near-infrared light (~800 nm wavelength) to optimize their growth yield and kinetics. Aquaculture being a rapidly growing industry needs a sustainable high quality feed substitute or incorporate with traditional protein-rich sources like soybean meal and fish meal. Two lab-scale 2-L photobioreactors were utilized in parallel to produce PPB with varying hydraulic retention time (HRT) and light availability to assess growth, productivity and composition.

A significant correlation between light exposure, HRT, and biomass growth response was found. As the HRT increased from 2-6 days, there was a significant decline in intracellular polyhydroxyalkanoates (PHA) accumulation, dropping from 11% (COD_{PHA}/COD_{VSS}) at 2-day HRT to 2.5% at 6-day HRT (light availability; 140 W/m^2). This suggests that shorter retention times favoured the storage of carbon as PHA at a constant organic loading rate (OLR equal to ~1.0 g $COD_{SOL}/L \cdot d$). Biomass productivity peaked at an HRT of 4 days under continuous light (0.31 g PPB/L·d), indicating an optimal balance between substrate availability and cell growth at this interval. It was also observed that 24 hours of consistent light gave better results in pigment production than 12-hour intermittent light. Total pigment content, including carotenoids (Crts) and bacteriochlorophylls (BChls) increased by increasing HRTs from 2 days (0.44 wt%) to 4 days (0.57 wt%), and remained approximately constant at 6 days HRT (0.59 wt%).Crts synthesis was also favoured by continuous light exposure while BChls showed an opposite trend. These results demonstrate that although longer HRTs may enhance certain growth yields, they differently impair the accumulation of high value bioproducts like PHA and pigments.

As possible utilization, if incorporated into fish feed at 20 wt%, this biomass can provide a functional PHA concentration of ~1.0-2.0 wt%, which serves as a natural antibiotic and health-promoting agent without the need for expensive PHA extraction processes. Hence, net of the safety aspect that need to be investigated, this approach may provide a sustainable winery residue management solution and a waste-to-value conversion route for aquaculture feeds.

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Integrating purple non-sulfur bacteria for agro-industrial waste valorization: optimization of biomass and hydrogen production

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The transition toward a circular bioeconomy demands biotechnological solutions capable of converting agro-industrial residues into high-value products while reducing environmental impacts. This work presents an integrated platform based on purple non-sulfur bacteria (PNSB), with a focus on *Rhodopseudomonas palustris*, developed within the SPIGA and WHISPER research projects.

The proposed biotechnology exploits the exceptional metabolic versatility of PNSB to valorize organic-rich waste streams through photofermentation and bio-electrochemical systems (BESs). Within the SPIGA project, appropriate pre-treatments of several agro-industrial wastes are evaluated, and a single-phase photofermentation platform is proposed to directly convert agro-industrial wastewater into green hydrogen (H_2), eliminating the conventional dark-fermentation step. Also, genetically engineered strains characterized by knocking out the uptake hydrogenase gene, overexpression of the hydrogen-evolving enzyme NifA*, and elimination of the Calvin-Benson cycle pathway, are tested to maximize H_2 yield even in the presence of ammonium, which is usually present at high concentrations in fermented organic wastes. In parallel, the WHISPER project integrates PNSB into multi-stage BESs designed for the treatment of agro-industrial effluents. In this configuration, the anodic oxidation of the effluents is coupled with a PNSB-based bio-photocathode. This integrated system enhances H_2 production and achieves a substantial reduction in chemical oxygen demand (COD). Importantly, the process increases biomass productivity compared to non-polarized systems.

Ensuring long-term stability under continuous-flow operation, optimizing electron transfer through conductive materials, managing variability in wastewater composition, scaling laboratory prototypes to industrially relevant volumes while remaining competitive with established waste treatment and energy technologies, and meeting regulatory requirements for the use of PNSB-derived biomass remain critical steps that must be addressed.

Overall, these PNSB-based platforms demonstrate strong market potential by enabling simultaneous organic-waste treatment, renewable energy generation, and the production of microbial biomass with further potential.

Acknowledgments:

This work was supported by Ministero italiano dell'Università e della Ricerca during the research program PRIN 2022 PNRR "Valorization of cheese whey by hydrogen production in bio-electrochemical systems catalyzed with purple bacteria (WHISPER)" and by Ministero dell'ambiente e della sicurezza energetica (MASE), Italy, Project SPIGA "Sviluppo di una piattaforma di Produzione di Idrogeno Green mediAnte sistemi innovativi, UGOV RSH2A_000025_SPIGA_PNRRM2.C2.I3.5".

Engineering Microbial Selectivity: Operational Strategies for PNSB-Based Resource Recovery in Open Systems

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While Purple Non-Sulfur Bacteria (PNSB) are premier candidates for converting wastewater into high-value microbial protein, the high cost of closed photobioreactors remains a major barrier to industrial scaling. This study investigates the use of open raceway reactors traditionally used for microalgae—as a cost-effective alternative. The primary challenge in open systems is maintaining high PNSB selectivity against competing aerobic heterotrophs.[1] We demonstrate that by strategically synchronizing chemical oxygen demand (COD) loading with light periods and suppressing nocturnal aeration, we can effectively "steer" the microbial community. Results show that maximizing the surface-to-volume ratio and eliminating dark-period substrate availability increased PNSB abundance to 78%, with protein productivities reaching 0.2 g L^{-1} day^{-1}. These findings provide a scalable operational framework for producing high-purity microbial feed ingredients within a circular bio economy.[2]

This study addressed the challenge of "selective pressure" in non-sterile open environments. In open systems, aerobic heterotrophic bacteria (AHB) often outcompete PNSB due to higher growth rates in the presence of oxygen. We implemented a multi-variable "steering" strategy focused on the Availability-Time-Light (ATL) nexus. By operating at a short 2-day sludge retention time (SRT) and utilizing a pulse-feeding regime, we synchronized the chemical oxygen demand (COD) loading rate with the maximum photosynthetic uptake capacity during daylight hours. To further suppress AHB, we eliminated nocturnal stirring, creating a transient anaerobic environment that starved aerobic competitors of oxygen and substrate simultaneously during the dark phase.

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PURPLE4LIFE 2026

SELECTED ABSTRACTS FOR ORAL PRESENTATIONS

DAY 2

PURPLEGAIN (CA21146)
PURPLE4LIFE (101212806)

WORKSHOP

"BOOSTING THE USE OF PPB AS INNOVATIVE FOOD AND FEED INGREDIENTS: FROM METABOLISM TO HEALTH-PROMOTING EFFECTS"

Day 2 – Session I

Improved color, health and nutrition of ornamental fish fed with purple bacteria

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Ornamental fish keeping is a rapidly expanding pet sector that can reach very high economic value, making nutrition-driven improvements in growth, appearance, and robustness commercially relevant. Conventional pigment boosters and fishmeal-dependent diets face sustainability and supply volatility concerns, motivating alternatives based on microbial sources [1]. Purple bacteria (PB) can be used as multifunctional microbial feed ingredients, combining high-quality protein with carotenoids and redox-active metabolites. In our lab we recently reported improved growth performance, pigmentation, and disease resistance in guppies [2] fed PB-based diets, indicating their potential as functional additives beyond conventional protein sources.

This new study evaluated the effects of the purple bacterium (*Rhodobacter Capsulatus*) as a feed additive for the economically important ornamental fish species koi (*Cyprinus rubrofuscus*). In a 12-week feeding trial, 400 fish were randomly assigned to five dietary treatments: a negative control diet without additives; a positive control diet supplemented with a pigment-rich *Chlorella* spp. and *Moringa oleifera* mixture; and experimental diets containing PB at 2%, 5%, and 10% inclusion levels. Growth performance was assessed through specific growth rate, total weight gain, and feed conversion ratio, while coloration was quantified using objective image-based colorimetric analysis (hue, saturation, and lightness). Ongoing work includes respirometry assays to evaluate metabolic performance, as well as cold-stress and pathogen-challenge tests to assess immune resilience together with targeted metabolomic analysis of plasma and liver samples.

Across treatments, PB supplementation resulted in superior growth performance (Figure 1). Diets containing PB achieved the highest specific growth rates and total weight gain, with 2% inclusion performing best overall, whereas the *Chlorella–Moringa* benchmark showed the weakest growth response. Color analysis revealed a distinct and commercially relevant pigmentation profile (Figure 2). PB supplementation significantly increased lightness, resulting in a visibly brighter appearance, a key quality trait in ornamental koi. In contrast, the *Chlorella–Moringa* diet intensified color saturation but produced darker fish. Together, these results position PB as a multifunctional and tunable feed additive capable of simultaneously improving growth and enhancing the most desirable visual trait in the ornamental market, while offering clear sustainability benefits through reduced reliance on fishmeal and plant-based or synthetic pigment sources.

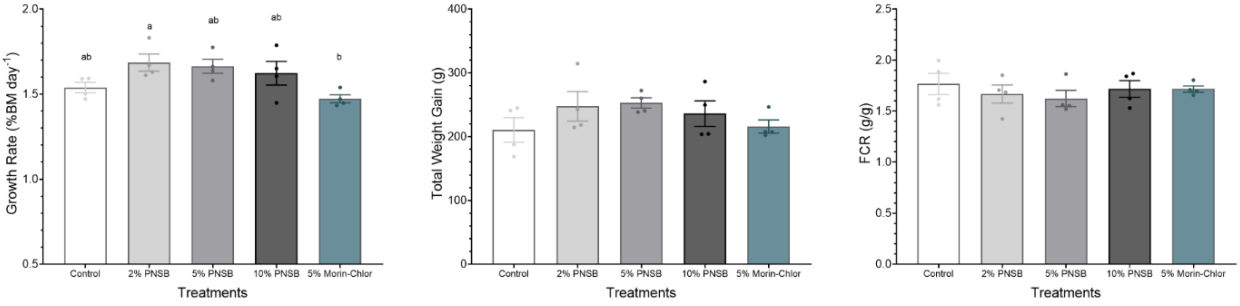

Figure 1. Growth performance of koi fed with control and treatment diets (12 weeks). Specific growth rate (% body weight per day), total weight gain (g), and feed conversion ratio (FCR, g g^{-1}) for five diets: control, 2% purple non-sulphur bacteria (PNSB), 5% PNSB, 10% PNSB, and 5% Chlorella–Moringa (Morin Chlor). Bars show mean with error bars, points show individual tanks (as plotted). Different letters indicate significant differences among treatments (one way ANOVA with post hoc multiple comparisons, $\alpha = 0.05$).

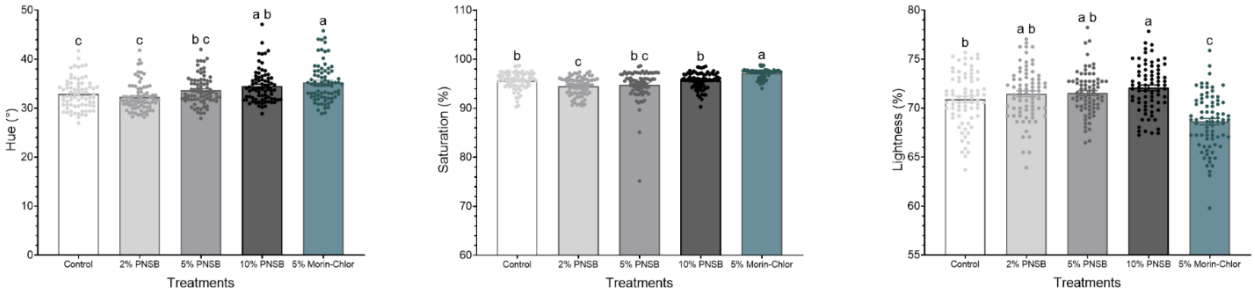

Figure 2. Colouration metrics of koi after 12 weeks of feeding. Hue (°), saturation (%), and lightness (%) extracted from standardized images (HSL space) for the same five dietary treatments. Points represent individual fish measurements and bars summarize central tendency with dispersion (as plotted). Different letters indicate significant differences among treatments (one way ANOVA with post hoc multiple comparisons, $\alpha = 0.05$).

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A dish with no fish: How DHA-free microalgae and linseed oil enhance n-3 LC-PUFA biosynthesis in rainbow trout (*Oncorhynchus mykiss*)

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Fish are a source of essential n-3 polyunsaturated fatty acids for human nutrition. In fish diets, fish oil provides EPA (20:5 n-3) and DHA (22:6 n-3) but sustainable alternative lipid sources are needed. Vegetable oils can contain n-3 short-chain PUFA (SC-PUFA) yet no n-3 long-chain PUFA (LC-PUFA). Unlike most saltwater fish species, rainbow trout can biosynthesise the LC-PUFA EPA and DHA if sufficient precursor fatty acids are provided [1]. The microalgae *Tetraselmis chui* contains no DHA but low amounts of EPA and additionally offers the precursor fatty acids ALA (18:3 n-3) and SDA (18:4 n-3). To test if *T. chui* could be a suitable fatty acid source in fish diets, a feeding trial with rainbow trout was conducted to evaluate growth performance and fatty acid utilization. Fish oil was partially substituted by the microalgae in fishmeal-free diets and linseed oil elevated the ALA content. Fish were fed daily with 1.9% of their body weight for 12 weeks followed by a digestibility trial. The algae diets reduced the growth performance of the fish at 14 % algae inclusion and decreased the diet digestibility, in accordance with other studies on microalgae [2,3]. No significant effect was observed regarding health parameters of the fish as well as no effect on the whole-body nutrient composition. Nevertheless, dietary microalgae and linseed oil increased biosynthesized DHA, underlining the ability of rainbow trout to produce this fatty acid from precursors [4]. The fillets of fish fed the algae diets demonstrated suitable DHA and n-3 levels but revealed a yellow pigmentation (Fig. 1). *T. chui* showed great potential as an alternative fatty acid source in fish nutrition although lacking in DHA. Understanding the physiological fatty acid pathways in fish is essential for future feed formulations and combining microalgae with vegetable oils could be a promising approach for sustainable feed formulations.

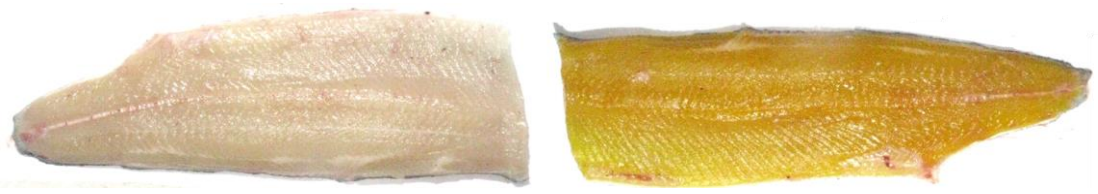

Figure 1. Fillet coloration of rainbow trout fed either fish oil diet (left) or microalgae diet (right) for 84 days

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Tailoring Egg Lipids Through Hen Nutrition: Human Health Effects and Opportunities for Novel Lipid Sources

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Eggs are among the most accessible and commonly consumed sources of animal proteins and lipids worldwide. Although eggs offer outstanding nutritional qualities, their lipid composition still has potential for further enhancement to better align with human dietary needs. Because hens use a significant part of the ingested fatty acids for deposition in the egg yolk, the egg's fatty acid profile is strongly influenced by diet, making eggs a particularly attractive matrix for nutritional modulation [1].

The essential fatty acid docosahexaenoic acid (DHA), consumed in amounts far below recommendations, along with less common fatty acids such as conjugated linoleic acids (CLAs) and conjugated linolenic acids (CLnAs), scarce in the human diet, have drawn significant interest due to their cardiometabolic, anti-inflammatory, and anticarcinogenic effects [2-6]. Promoting food sources capable of efficiently incorporating these fatty acids is therefore particularly valuable [7-8].

Sixty-six hens were therefore fed for twenty-six weeks, either a control diet rich in oleic acid or a test diet enriched with flaxseed oil (high in linolenic acid, ALA, a DHA precursor) and pomegranate seed oil (rich in punicic acid, PunA, a CLnA and precursor of rumenic acid, RmA, a CLA). Eggs produced under both conditions were subsequently used in a double-blind randomized controlled trial involving twenty-four adults with abdominal obesity, who consumed two eggs per day for three months.

Dietary modulation of hens significantly increased yolk levels of ALA, DHA, PunA, and RmA without affecting egg production and weights [9]. Moreover, the human intervention study showed that consumption of enriched eggs was associated with a significant reduction in waist circumference compared with control eggs [10].

This work demonstrates the feasibility of tailoring egg lipid composition through targeted feeding strategies to enhance the nutritional value of a widely consumed food, leading to measurable human health benefits. These results open perspectives for incorporating alternative lipid sources, including lipid extracts from purple phototrophic bacteria (PPB), which are rich in monounsaturated fatty acids (such as cis-vaccenic acid), a class of lipids associated with reduced cardiovascular risk factors [9-10]. PPB also contain antioxidant compounds, including carotenoids and coenzyme Q10, which have been linked to protective effects against atherosclerosis and atherogenic dyslipidemia [11-13].

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Diet composition and environment interact and control flesh color in salmon

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Salmonids can deposit xanthophylls (oxygen-containing carotenoids) like astaxanthin and canthaxanthin in their muscle, which give them their red flesh color. The fillet color is an important quality trait for farmed Atlantic salmon, but there have been concerns about paler fillet color [1], but increasing the diet content of astaxanthin has not solved the problem (Figure 1). Pale fillet color has been hypothesized to be stress related. Mechanical methods for removal of salmon lice are stressful for the fish, and such treatments have increased in frequency in the last decade. Astaxanthin is a strong antioxidant that quenches free radicals and singlet oxygen [2] and oxidative stress could potentially break down the astaxanthin into short-chain colorless compounds. Thus, the use of novel dietary antioxidant products such as CoQ10 from PPB can potentially protect astaxanthin from degradation and improve fillet pigmentation, particularly during stressful conditions. Even during optimal conditions, fillet deposition of astaxanthin is not very efficient, only around 5-10% of the ingested astaxanthin is retained in the muscle. The digestibility of astaxanthin is also quite low and varies considerably with diet composition. Carotenoids are lipid soluble compounds, and their absorption in the intestine is dependent on the lipid content and composition of the diet. We have also seen that the metabolic conversion of astaxanthin is affected by dietary components. The origin of the ingredients in the salmon diet has changed considerably in the last decades, from marine to mainly plant ingredients [3]. This has led to lower dietary levels of several nutrients (phospholipids, vitamin A, omega-3 fatty acids, cholesterol) that can affect both absorption and metabolism of astaxanthin in the salmon [4,5,6]. Nofima has worked together with the industry with understanding how dietary and environmental mechanisms interact and control flesh pigmentation in salmon, and some of our latest findings will be presented at the work-shop.

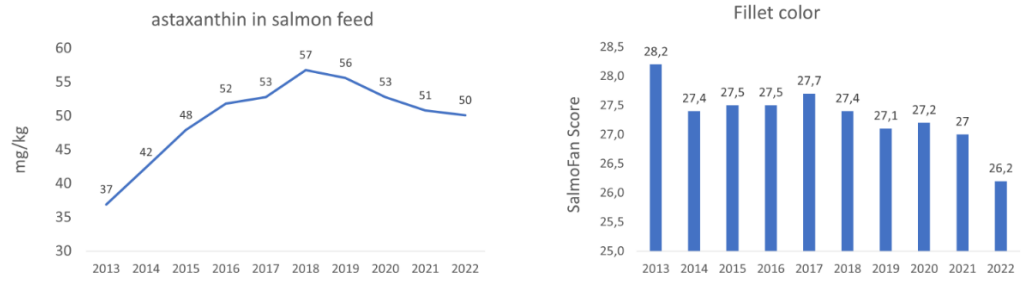

Figure 1. Development in content of astaxanthin in salmon feeds and fillet color from 2013 to 2022 in Norwegian salmon farming (Data from commercial farming in Norway)

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Valorisation of Food Industry By-Products for Single-Cell Protein Production

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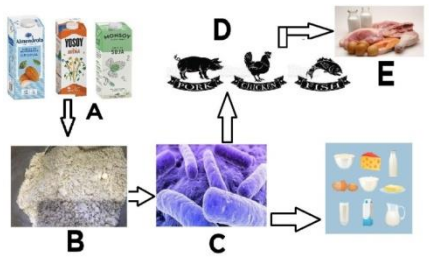
Abstract. Single-Cell Protein (SCP) from fungi, algal and bacterial sources is perspective alternative component to meet growing shortage of protein sources in the world. SCP production has multiple advantages over conventional food production practices such as: microorganisms have a high rate of multiplication, which means a large quantity of biomass can be produced in a comparatively shorter duration. The raw materials for SCP production based on waste substances are cheap, readily available and their processing contributes to reducing environmental pollution. The research includes the culture of PNSB over plant based beverage`s by-products at a laboratory scale reactor. The protein-rich biomass was compared to commercially available protein sources to underpin the potential of replacing these products with recovered SCP. Project results shown that SCP, produced by purple bacteria from by beverage by-products, is very perspective protein source for broiler and swine feed production. Further research is needed.



ESSENTIAL AA % of total protein	Thr	Cys	Val	Met	Ile	Leu	Phe	Lys
			OWN RESEARH					
Protein sources								
GM	4,44	0,31	2,20	0,92	1,44	2,85	3,86	3,04
SM	1,71	0,61	3,83	1,94	2,62	5,07	1,70	1,56
CM	4,11	0,83	4,99	2,57	3,28	6,57	3,78	3,82
CA	4,70	0,98	5,92	2,99	3,86	7,38	4,48	4,32
Soy	0,77	0,47	0,72	0,28	0,60	1,16	0,75	1,01
Mix Cereal	1,25	0,93	1,85	0,71	1,26	2,89	2,16	1,42
Traditional protein sources								
Sunflower meal CP 31.48[a]	1.29	0.61	1.73	0.81	1.45	2.21	1.57	1.26
Soybean meal CP48,1	1.88	0.71	2.28	0.67	2.21	3.73	2.45	3.01
Fish meal CP 61.85[a]	2.59	0.53	3.08	1.77	2.59	4.56	2.49	4.8
Rapeseed meal CP 32.96[a]	1.42	0.74	1.6	0.64	1.26	2.37	1.36	1.75

Table 1. Amino acid composition of produced SCP compared with sunflower meal / soybean meal / fish meal

The results presented in Table 2 demonstrate clear differences in the amino acid composition of single-cell protein (SCP) produced from different substrates. SCP derived from GM, SM, CM and CA was compared with conventional protein sources commonly used in animal feed production. The analysis showed that SCP contained high levels of essential amino acids required for animal nutrition, particularly lysine and methionine, which are often the first limiting amino acids in broiler, swine and ruminant diets.

Lysine plays a critical role in supporting growth, milk production and overall animal health, while methionine is considered the primary limiting amino acid in poultry diets and one of the major limiting amino acids in swine nutrition. SCP produced from GM, CM and CA contained higher lysine concentrations than soybean, sunflower and rapeseed meals. Similarly, methionine levels in SCP from GM, SM, CM and CA exceeded those found in conventional plant protein sources.

Overall, SCP produced by purple non-sulphur bacteria demonstrated a more balanced essential amino acid profile compared to soybean meal, with higher concentrations of several key amino acids. These results indicate that SCP derived from food by-products represents a promising alternative protein ingredient for broiler and swine feed, although further studies on digestibility and in vivo performance are required.

Day 2 – Session II

Vitamin B12 production in Purple Non-Sulfur Bacteria: Impact of autotrophy versus heterotrophy and anaerobic versus aerobic conditions

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Cobalamin (vitamin B12) is an essential micronutrient for humans and animals, synthesised exclusively by certain bacteria and archaea [1]. Purple non-sulfur bacteria (PNSB) represent a promising source of cobalamin, yet research on their production remains limited. A major challenge in cobalamin quantification is the reliance on cyanide-based methods, which are hazardous and require special certifications. In this study, we tested a novel extraction and quantification approach for PNSB biomass, replacing cyanide with sodium metabisulfite to convert cobalamin into a stable sulfonated form [2], followed by HPLC analysis.

Cobalamin production (at pH 7, 30 °C) by *Rhodobacter (Rb.) capsulatus* was first screened in flask cultures under heterotrophic (acetate) and autotrophic (H_2/CO_2) conditions, across three cobalt supplementation levels (0, 5, and 10 µM). The optimal condition, heterotrophic growth with 5 µM cobalt, was then to *Cereibacter (Cb.) sphaeroides* and *Rhodopseudomonas (Rps.) palustris* to assess interspecies variability in cobalamin production. Subsequently, *Rb. capsulatus* was cultivated in a bioreactor under semi-continuous heterotrophic conditions (10 µM cobalt) at a dilution rate of 1 d^{-1}, both anaerobically and under aerobic conditions, maintaining in the latter case dissolved oxygen levels at 5% saturation.

No cobalamin was detected without cobalt in the growth medium, and concentrations were similar at 5 and 10 µM Co (Figure 1A), indicating no further benefit from higher cobalt supply within the tested range. The highest cobalamin content in flask cultures was 5.1 ± 0.3 mg·100 gDW^{-1} under photoheterotrophic conditions. When the selected conditions were applied across species, *Cb. sphaeroides* and *Rps. palustris* accumulated 4.3 ± 0.1 and 4.5 ± 0.2 mg·100 gDW^{-1}, respectively, showing cobalamin levels marginally lower yet comparable to those obtained with *Rb. capsulatus*. In semi-continuous bioreactor operation, cobalamin levels were lower: 2.6 ± 0.4 mg·100 gDW^{-1} but increased by 36% under aerobic conditions (Figure 1B). Cobalamin concentrations in PNSB biomass are significantly higher than what is found in conventional sources, namely animal-derived products, which contain around 0.2 to 60 µg 100gDW^{-1}[1]. These findings support the use of PNSB biomass as a vitamin B_{12}–containing ingredient for food and feed applications.

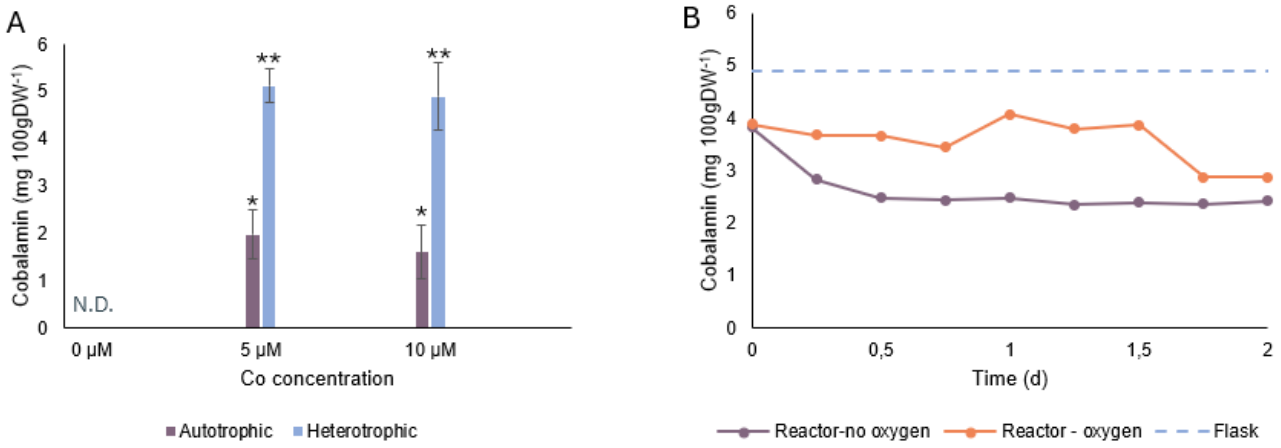

Figure 1. A) Cobalamin concentration in *Rb. capsulatus* under autotrophic and heterotrophic conditions. 'N.D.' stands for 'not detected'. Asterisks above the bar plots indicate statistically different results (p-value < 0.05). B) Cobalamin concentration in *Rb. capsulatus* cultivated in a bioreactor under heterotrophic conditions with and without oxygen, at 10 µM of cobalt. The horizontal dashed line is the content reach in the heterotrophic flask-based experiment at 10 µM cobalt (4.9 mg/100 g DW).

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Lipid extraction and Coenzyme Q_{10} quantification in purple phototrophic bacteria.

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Work objective and key challenges

We have developed a lipid extraction method from purple phototrophic bacteria (PPB), which is highly reliable and reproducible and has been optimized for the quantification of Coenzyme Q10 (CoQ_{10}) and other antioxidant compounds.

Methodology

- Homogenization by sonication for 5 minutes (1 mg PPB biomass in 0.2 mL PBS). Add 20 µL CoQ_6 (200 µM) as internal standard to calculate the extraction yield.
- Lipid solubilization with 0.4 mL ethanol:isopropanol (95:5) and vigorous shacking.
- Lipid extraction (3 times) with 1 mL hexane, vigorous shacking, and centrifugation. Collect and mix the 3 upper layers and evaporate the hexane in a vacuum centrifuge at 40 °C.
- Reconstitute the extract in 1 mL of methanol:1-propanol (85:15).
- Determine CoQ_{10} concentration by HPLC with UV (275 nm) and electrochemical detectors (reduction cell at -700 mA, oxidation cell at +500 mA and reading at 5 Hz for 18 minutes). HPLC conditions [1]: C18 column (5x150x4.6 mm) at 40ºC, flow rate at 1,2 mL/min (85% metanol:ammonium acetate 98:2, 15% 1-propanol:ammonium acetate 98:2) for 9 minutes + 9 minutes at 1,1 mL/min (50% metanol:ammonium acetate 98:2, 50% 1-propanol:ammonium acetate 98:2).

Results

Each PPB strain exhibits a characteristic lipid profile (Figure 1). All PPB strains contain high amounts of CoQ_{10} (Table 1). The fatty acid composition is also specific to each PPB (Table 2).

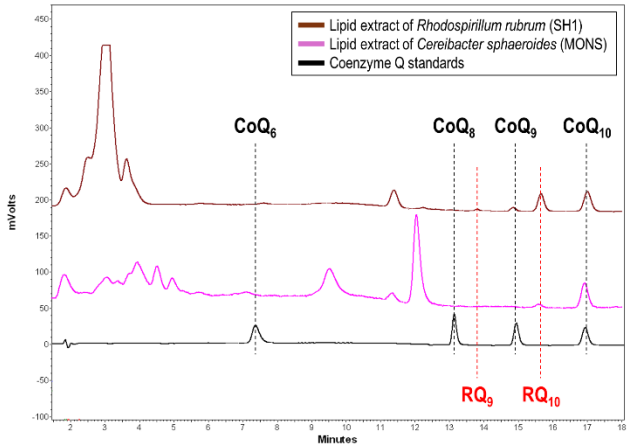

Figure 1. Detection of lipids with redox activity using HPLC coupled to an electrochemical detector. Commercial CoQ standards (black).

Table 1. Coenzyme Q and Plastoquinone content in PPB (mg / g dry biomass)

PPB strain	CoQ_{10}	CoQ_9	PQ_{10}
1 - Rhodopseudomonas palustris (TIE1)	**2.1 ± 0.1**	**0.88 ± 0.18**	n.d.
2 - Rhodopseudomonas palustris (420L)	**2.3 ± 0.2**	**0.46 ± 0.06**	n.d.
3 - Rhodospirillum rubrum (SH1)	**4.8 ± 0.3**	**0.68 ± 0.07**	**2.36 ± 0.10**
4 - Cereibacter sphaeroides (MONS)	**4.2 ± 0.1**	**0.22 ± 0.01**	**0.68 ± 0.02**
5 - Cereibacter sphaeroides (ANTWERP)	**4.0 ± 0.4**	n.d.	n.d.
6 - Rhodobacter capsulatus (ANTWERP)	**4.0 ± 0.2**	n.d.	**1.29 ± 0.55**

Table 2. Fatty acid composition in PPB

PPB strain	Caprylic C8:0	Palmitic C16:0	Palmitoleic C16:1	Stearic C18:0	Oleic C18:1n9c	Linoleic C18:2n6c
1 - Rhodopseudomonas palustris (TIE1)	n.d.	**13%**	**3%**	**14%**	**65%**	n.d.
2 - Rhodopseudomonas palustris (420L)	n.d.	**14%**	**3%**	**13%**	**63%**	n.d.
3 - Rhodospirillum rubrum (SH1)	n.d.	**15%**	**22%**	**1%**	**38%**	**13%**
4 - Cereibacter sphaeroides (MONS)	**15%**	**7%**	**4%**	**10%**	**60%**	n.d.
5 - Cereibacter sphaeroides (ANTWERP)	**10%**	**7%**	**1%**	**13%**	**64%**	n.d.
6 - Rhodobacter capsulatus (ANTWERP)	n.d.	**4%**	**7%**	**5%**	**80%**	n.d.

Conclusion.

PPBs are a natural source of CoQ_{10}, fatty acids and other antioxidant compounds, some not yet identified, and could be used as a nutritional supplement. It is urgent to analyze the bioavailability of this CoQ and its incorporation through diet in humans or animal models.

Potential market impact and sustainability benefits

- 20-75 grams PPB biomass could provide the pharmacological dose of CoQ_{10} for the treatment of mitochondrial diseases (100-300 mg CoQ_{10}/day).
- 6-7.5 grams PPB biomass could provide the amount of CoQ_{10} as a nutritional supplement (30 mg of CoQ/day).

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Purple phototrophic bacteria as a sustainable protein source: bioaccessibility and functional responses during in vitro digestion

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The global food system faces increasing environmental pressure, while growing recognition of the importance of diet-related gut health is reshaping nutritional priorities. Purple phototrophic bacteria (PPB) represent a promising microbial resource, enabling carbon-efficient production of protein-rich biomass with potential health benefits. However, the bioaccessibility of PPB-derived proteins and bioactive compounds during digestion, and the influence of pretreatment on their release and activity, remain poorly understood.

Freeze-dried *Rhodobacter capsulatus* was subjected to high-pressure homogenization (HPH), sonication, or solvent extraction, after which antioxidant-, carotenoid-, and protein-associated characteristics in the resulting supernatants were spectrophotometrically assessed. HPH consistently produced the highest assay signals and was identified as the most effective pretreatment. Further size fractionation and correlation analyses suggested liberation of structurally stabilised carotenoid-protein assemblies (>100 kDa), possibly chromatophore-like structures, explaining the activity of these lipophilic antioxidants in the aqueous supernatants.

In vitro digestion of HPH-treated and untreated samples, characterised by protein profiling and spectrophotometric assays, revealed progressive protein hydrolysis and enhanced carotenoid-associated availability in the intestinal phase, both more pronounced following HPH pretreatment. Furthermore, polysaccharide-containing structures persisted throughout digestion, together indicating potential for uptake and local gut interactions of PPB-derived components.

Preliminary cellular assays using undifferentiated Caco-2 cells demonstrated concentration-dependent biological responses. At higher biomass concentrations, decreased cellular protein content and growth were likely driven by prooxidative carotenoid activity and digestion matrix-related cytotoxicity, whereas growth-enhancing effects emerged upon dilution, appearing at higher concentrations of untreated PPB and only after further dilution of HPH-treated biomass.

Overall, the results indicate that PPB biomass may act as a natural encapsulation for bioactive compounds, enabling intestinal-phase delivery, while modulation of HPH intensity offers opportunities to tailor gastrointestinal release profiles towards specific functional objectives. As one of the first studies to explore PPB bioaccessibility and cellular effects, this thesis sheds new light on the relationships between pretreatment, structural stabilization, and functional outcomes in PPB, supporting their development for future sustainable health-promoting food solutions.

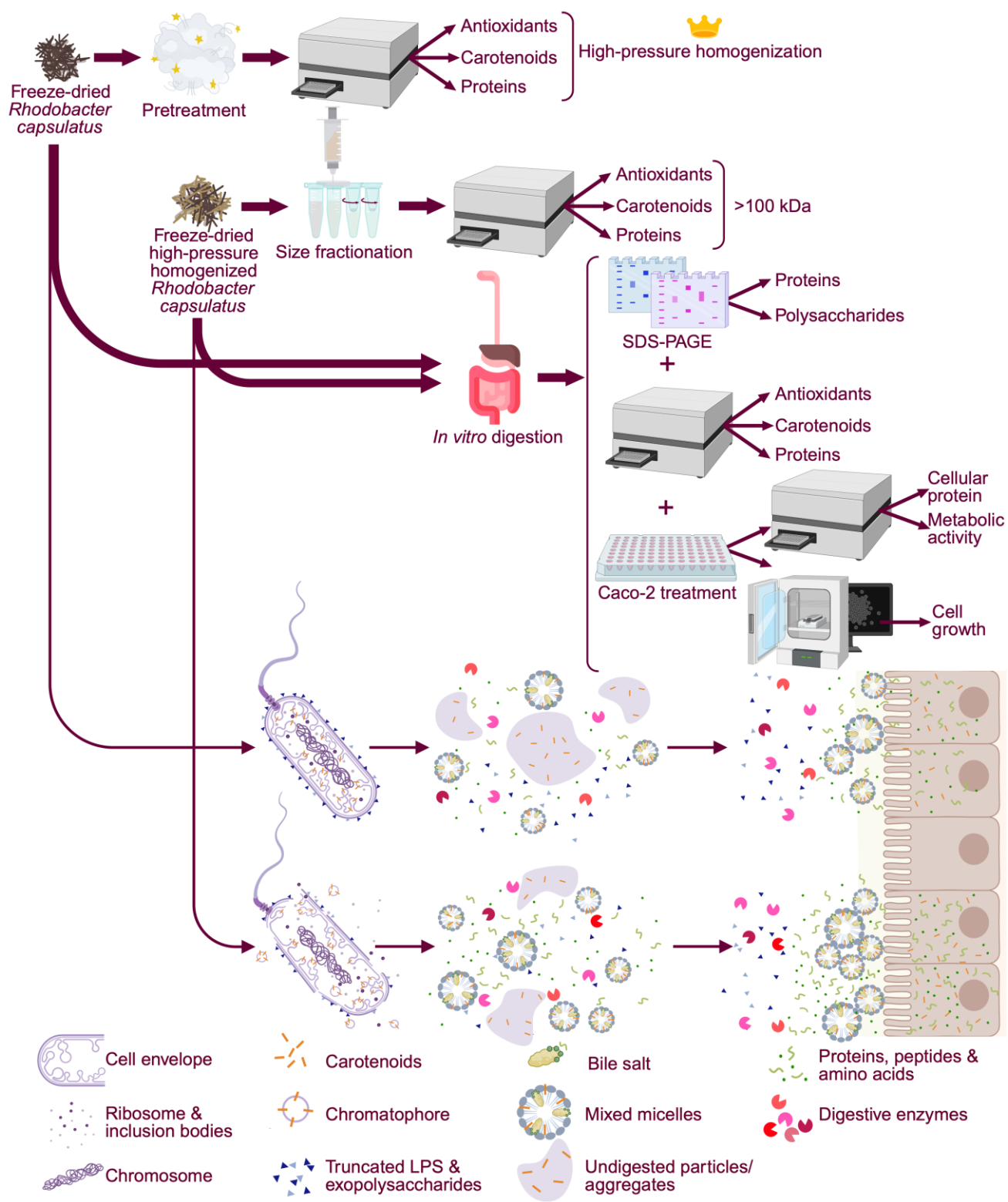

Figure 1. Graphical abstract illustrating the experimental workflow and schematic overview of the proposed assemblies following *Rhodobacter capsulatus* pretreatment and *in vitro* digestion, and their potential interactions with intestinal cells. Created using BioRender.

Optimising spraydrying to maximise antioxidant capacity in purple bacterial biomass

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Meeting future protein and health-promoting compound demand requires scalable and resource-efficient microbial food platforms. Purple phototrophic bacteria (PPB) represent a promising option, combining protein-rich biomass with the biosynthesis of bioactive antioxidants, notably carotenoids and coenzyme Q10, thereby enhancing nutritional value and shelf-life stability. Preserving these heat- and oxygen-sensitive compounds during downstream processing, however, remains a key challenge [1].

Among processing steps, biomass drying constitutes a major technical and economic bottleneck, accounting for up to 60% of total process energy demand while strongly influencing product stability and shelf life. Following harvesting and concentration, PPB biomass is obtained as a wet paste containing 5–15% solids and must be rapidly processed to limit microbial and oxidative degradation. Conventional drying technologies, such as oven drying and freeze drying, involve trade-offs between energy demand, processing time, and compound preservation, limiting their industrial applicability [2].

Spray drying offers high throughput and relatively low operating costs while enabling rapid moisture removal and powder stabilisation. Importantly, it can be tailored to balance process efficiency with the preservation of carotenoids and CoenzymeQ10, key contributors to antioxidant capacity and shelf-life stability [3]. To date, the impact of spray drying on PPB biomass, particularly regarding antioxidant retention and shelf-life stability, has not been evaluated.

In this study, spray drying of PPB biomass is systematically investigated to maximise yield while preserving antioxidant functionality. Response surface methodology based on a central composite design was applied to assess the effects of inlet air temperature (80–150 °C), feed solids content (5–20% w/w), and feed rate (10–40 mL min^{-1}), at constant atomization pressure (1 bar), on drying performance, residual moisture, and retention of carotenoids and CoenzymeQ10. Spray-dried biomass was benchmarked against oven-dried and freeze-dried samples, and shelf-life degradation kinetics were evaluated under storage at −20, 4, and 20 °C, under normal atmosphere and vacuum.

At this stage, initial spray-drying runs were successful and data processing is ongoing. Preliminary tests indicate literature-consistent trends, with inlet air temperature governing drying efficiency and powder recovery, while feed solids content supports antioxidant retention by mitigating thermal and oxidative stress. Full results will be available at the workshop.

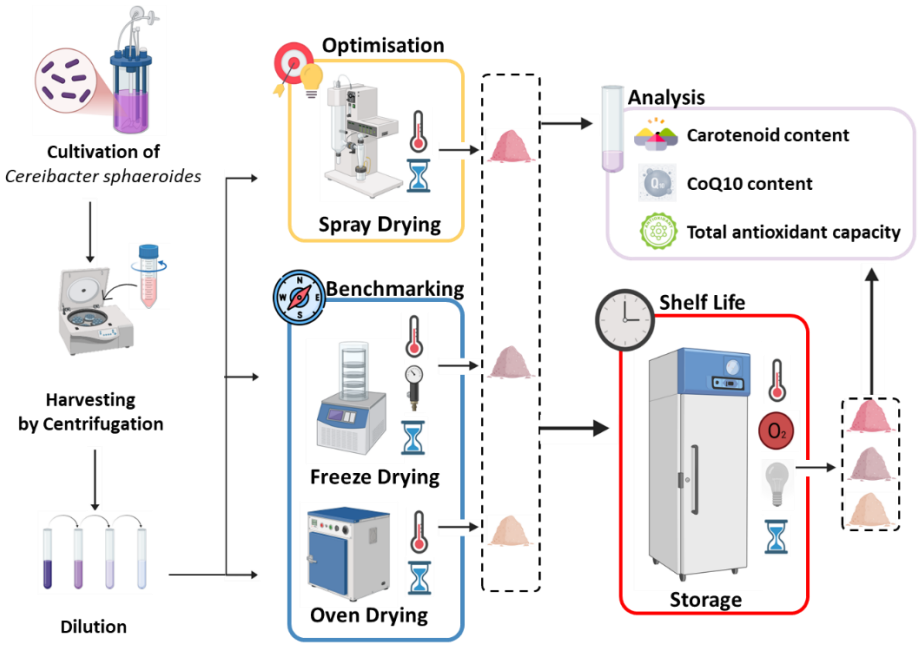

Figure 1. Experimental workflow for drying and shelf-life assessment of purple phototrophic bacterial biomass

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PURPLE4LIFE 2026

ABSTRACTS SELECTED FOR POSTER

DAY 2

PURPLEGAIN (CA21146)
PURPLE4LIFE (101212806)

WORKSHOP

"BOOSTING THE USE OF PPB AS INNOVATIVE FOOD AND FEED INGREDIENTS: FROM METABOLISM TO HEALTH-PROMOTING EFFECTS"

Upcycling Food Waste into Active Biopolymer Films and Exploring Their Integration with PPB-Based Photorefineries

Sehabat Öztekin

Food processing generates a surprising amount of phenolic-rich residues, from peels and seeds to various forms of pomace, yet much of this material is still overlooked despite its clear potential within circular valorisation strategies. Growing interest in sustainable material development has shown that these by-products can be transformed into valuable functional ingredients, including natural antioxidants, colour-giving pigments, and carbon-based nanostructures such as carbon quantum dots. When these components are incorporated into biodegradable polymer matrices, they contribute to the formation of active films with antimicrobial properties and colour-responsive behaviour, offering a promising way to support the preservation of highly perishable fruits. The present study focuses on valorising rosehip waste, onion peel, grape pomace, and similar agro-industrial residues as sources of functional additives for biodegradable packaging. Early results highlight that both natural extracts and carbon quantum dots obtained from these residues can enhance film performance by strengthening antioxidant capacity, providing light-driven antimicrobial effects, and enabling colour shifts that respond to pH changes associated with spoilage. These features point to a strong potential for reducing postharvest losses in soft fruits such as citrus and strawberries, which are particularly prone to fungal deterioration. Beyond their immediate application in food preservation, these waste-derived materials also offer meaningful points of connection with purple phototrophic bacteria (PPB)-based photorefineries. In future circular systems, liquid fractions of food waste could be channelled into PPB bioprocesses, while solid fractions could be used to produce active biopolymer films. This complementary pathway supports the creation of closed-loop value chains that bring together waste valorisation, microbial conversion, and biodegradable packaging. Overall, the work illustrates how food residues can be upgraded into high-value materials while aligning with emerging PPB technologies.

Exploring Purple Phototrophic Bacteria as a Sustainable Source of CoQ10 for Functional Foods

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Purple phototrophic bacteria (PPB) are a promising natural source of bioactive compounds, including Coenzyme Q10 (CoQ10) and carotenoids, with potential applications in functional foods and nutraceuticals. This study provides a literature-based assessment of PPB metabolic capabilities, cultivation strategies, and product recovery methods to evaluate their suitability for sustainable CoQ10 production. Key aspects such as CoQ10 yield, biomass productivity, extraction approaches, and product stability are analyzed across reported studies. Regulatory considerations for food-grade applications and scalability challenges are also discussed to identify critical barriers for industrial adoption. The review highlights opportunities to optimize cultivation conditions and metabolic pathways using theoretical and model-based approaches, aiming to maximize CoQ10 content while maintaining biomass quality. Anticipated market impacts include providing a naturally derived, bioavailable CoQ10 source to meet growing consumer demand for health-promoting ingredients, supporting cardiovascular and antioxidant health. By synthesizing current knowledge, identifying research gaps, and exploring potential commercialization pathways, this study underscores the feasibility and sustainability of PPB as a platform for high-value functional food ingredients. Participation in the workshop will facilitate interactions with industry experts, knowledge exchange on regulatory and market aspects, and discussions on collaborative strategies to accelerate adoption of PPB-based CoQ10 solutions. Overall, PPB represent a versatile and environmentally sustainable microbial platform for producing bioactive compounds that can contribute to the development of functional foods and nutraceuticals while supporting circular economy principles.

Role of Bio-Priming with Purple phototrophic bacteria and 2,3-dihydroxybenzoic Acid in Enhancing Wheat Germination and Seedling Growth Under Salinity Stress

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Purple phototrophic bacteria (PPB) are emerging as promising biostimulants for enhancing plant resilience under abiotic stress conditions. They have vital phototrophic, chemotrophic, and diazotrophic capabilities. When applied to agricultural systems through seed coating, soil inoculation, or foliar sprays, PPB can improve plant tolerance to salinity, drought, heavy metals, and oxidative stress. Their ability to fix atmospheric nitrogen, solubilize phosphates, and enhance micronutrient availability directly supports plant nutrition under stress-impaired conditions. Under salinity stress, PPB can improve ion homeostasis by reducing sodium uptake and enhancing potassium assimilation. 2,3-dihydroxybenzoic acid (2,3-DHBA), which is a salicylic acid (SA) derivative, also supports plant growth against salinity stress.

The purpose of our study is to provide a priming application with PPB and 2,3-DHBA to tolerate salinity stress in wheat. Effects of six different irrigation water salinity (0.38, 2, 4, 7, 10, 15 dS/m) and three different priming practices (control, PPB and PGPR) on antioxidant enzyme activity, malondialdehyde (MDA) levels, proline contents, photosynthetic capacity and growth characteristics were investigated. Salt stress caused a decrease in germination rate, tillering number, yield parameters, stomata, chlorophyll and carotenoid contents. Among the treatments, PPB increased the germination rate and fresh weight by 6.30% and 14.20%, respectively, compared to the control. In addition, PPB increased catalase, ascorbate peroxidase, glutathione reductase and superoxide dismutase enzyme activities by 19.25%, 13.02%, 61.77% and 18.35%, respectively, while 2,3-DHBA only increased guaiacol peroxidase activity by 8%. PPB also decreased MDA content by 27.43% and increased proline content by 24.43%. PPB application more effectively alleviated germination, plant growth and development in high salinity conditions. Thus, it was concluded that using PPB together with plant growth regulators might be beneficial in order to tolerate salinity stress for different plants.

Keywords: *Abiotic Stress, Seed Priming, Purple phototrophic bacteria, Antioxidant Enzyme*

Designing with Living Color: Purple Phototrophic Bacteria in Sensorial and Emotional Culinary Design

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Abstract

Purple Phototrophic Bacteria (Purple PPB) represent an emerging and innovative category of food and feed ingredients that extend beyond nutritional and metabolic value to offer new possibilities for sensorial, emotional, and experiential food design. Within the framework of circular design and sustainable culinary practices, Purple PPB enable a rethinking of how ingredients are produced, perceived, and emotionally experienced by users and consumers.

From a circular metabolism perspective, Purple PPB efficiently transform organic by-products and residual biomass into high-value ingredients through low-energy, bio-based processes. This regenerative production model supports closed-loop food systems and aligns with circular design principles, while providing a material basis for innovative culinary expressions rooted in sustainability.

Beyond their metabolic efficiency, Purple PPB possess distinctive chromatic, textural, and aromatic properties derived from their natural pigments and bioactive compounds. These sensory characteristics open new design opportunities for chefs and food designers to explore color, visual identity, mouthfeel, and flavor modulation, fostering emotional connections between consumers and sustainable food products. The natural purple hues of PPB, for instance, can act as emotional triggers, conveying meanings related to nature, vitality, and innovation, while enhancing product storytelling and user engagement.

Moreover, the integration of Purple PPB into culinary design supports the creation of health-promoting experiences, where functionality and pleasure coexist. Their antioxidant and bioactive profiles contribute to well-being, while sensorially driven design strategies encourage acceptance and positive emotional responses to novel bio-based ingredients.

By positioning Purple PPB at the intersection of metabolism, health, sensorial design, and emotional experience, this approach highlights their potential to reshape sustainable food systems—not only through circular efficiency, but also by redefining how food is felt, perceived, and valued by society.

Extraction, biochemical and technofunctional characterization of proteins and phycocyanin **from *Phormidium versicolor*: Functional, Nutritional, and Application Potential**

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University of Sfax, High Institute of biotechnology of Sfax, Food Technology departement, Sfax, Tunisia

Abstract

Proteins and C-phycocyanin extracted from *Phormidium versicolor* were evaluated for their nutritional, structural, and technofunctional properties. The protein isolate exhibited a high water-holding capacity (3.8 g water/g), good oil-holding capacity, excellent emulsifying activity at low concentrations (1–2%), and a remarkable foaming capacity reaching 221%, with high foam stability, supporting its application in emulsified and aerated food systems. Nutritional analysis revealed a balanced essential amino acid profile, with amino acid scores exceeding reference values for both adults and children, along with good in vitro digestibility and Hazard and Risk Index values well below safety thresholds.

Purified C-phycocyanin retained its structural integrity, as confirmed by SDS-PAGE (α ~17 kDa, β ~19 kDa) and FTIR analysis, and showed an amorphous structure (XRD) favorable for solubility. Thermal analyses (TGA/DSC) indicated typical protein–pigment stability. Functionally, C-phycocyanin displayed moderate water-holding capacity, good oil-holding capacity, and excellent emulsifying activity at 1–2%, with increased stability at higher concentrations. Overall, these results highlight *P. versicolor* as a safe, nutritionally valuable, and multifunctional microalgal resource for sustainable food and nutraceutical applications.

Keywords: *Phormidium versicolor, proteins, phycocyanin, essential amino acids, digestibility, technofunctional properties, food applications.*

Valorization of Olive Mill Wastewater Using Purple Phototrophic Bacteria: A Review

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Olive mill wastewater (OMW) is one of the most problematic agro-industrial effluents due to its high organic load, acidity, and elevated concentrations of phenolic compounds, which cause severe environmental concerns. At the same time, OMW is rich in valuable organic carbon and bioactive molecules, making it an attractive substrate for biotechnological valorization. In recent years, purple phototrophic bacteria (PPB), particularly purple non-sulfur bacteria, have gained attention as versatile microorganisms capable of converting organic-rich waste streams into value-added products. This review summarizes current research on the utilization of OMW as a substrate for PPB growth and metabolism. Previous studies have demonstrated that PPB can grow on diluted or pretreated OMW under photoheterotrophic conditions, leading to the production of protein-rich microbial biomass, biohydrogen, and biopolymers such as poly-β-hydroxybutyrate. Although the high phenolic content of OMW may inhibit bacterial growth, appropriate pretreatment strategies—including dilution, pH adjustment, and partial phenol removal—have been shown to significantly improve PPB performance. Beyond biomass generation, PPB metabolism enables the biotransformation of phenolic compounds, reducing wastewater toxicity while potentially enhancing the bioavailability of phenol-derived metabolites. In addition, PPB biomass contains high levels of proteins, pigments (carotenoids and bacteriochlorophylls), and antimicrobial compounds, highlighting its potential as an innovative ingredient for food and feed applications. Overall, the integration of PPB-based processes into olive oil production chains represents a promising circular bioeconomy approach. Future research should focus on optimizing cultivation conditions, improving downstream extraction of valuable components, and evaluating the safety and functionality of PPB-derived products. Such advances could enable the sustainable conversion of OMW from an environmental burden into a valuable resource for food, feed, and health-promoting applications.

Keywords: *olive mill wastewater, purple phototrophic bacteria, bacterial growth, protein, pigment*

Purple Phototrophic Bacteria in Sustainable Food and Feed Production: Nutritional Value, Bioactive Compounds, and Health Effects

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Purple phototrophic bacteria (PPB) have recently gained increasing attention as sustainable and innovative resources for food and feed production due to their unique metabolic flexibility and rich nutritional profile. These bacteria are capable of utilizing a wide range of organic and inorganic substrates under phototrophic and chemotrophic conditions, enabling efficient biomass production from agro-industrial residues and wastewater streams. This characteristic positions PPB as promising candidates within circular economy and sustainable bioprocessing frameworks.

PPB biomass is characterized by high protein content, balanced amino acid composition, and the presence of valuable micronutrients, including vitamins, minerals, and essential fatty acids. In addition to their nutritional value, PPB are recognized for producing a variety of bioactive compounds such as carotenoids, bacteriochlorophylls, polyphenol-like substances, and extracellular polysaccharides. These compounds exhibit significant antioxidant, anti-inflammatory, antimicrobial, and immunomodulatory activities, contributing to the health-promoting potential of PPB-derived ingredients.

Recent studies have demonstrated the applicability of PPB in animal feed, particularly in aquaculture and livestock production, where their inclusion has been associated with improved growth performance, enhanced feed efficiency, modulation of gut microbiota, and increased resistance to oxidative stress and pathogenic challenges. Emerging evidence also highlights the potential of PPB as functional food ingredients, offering benefits related to metabolic health, immune function, and oxidative balance. Moreover, the natural pigment profile of PPB provides opportunities for their use as clean-label colorants with added functional properties.

Despite these promising attributes, challenges related to large-scale cultivation, downstream processing, safety assessment, and regulatory approval remain to be addressed. This review summarizes current knowledge on the nutritional composition, bioactive compounds, and health effects of purple phototrophic bacteria, while also discussing technological and regulatory considerations for their integration into sustainable food and feed systems. Overall, PPB represent a multifunctional and environmentally friendly bioresource with significant potential to contribute to future food security and health-oriented nutrition strategies.

Keywords: *Purple phototrophic bacteria, Sustainable food and feed, Bioactive compounds, Alternative proteins*

CRISPR-Cas9-mediated genome integration of a fluorescent reporter enables single-cell analysis of nitrogenase expression in Rhodospirillum rubrum

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Microbial bioprocesses are commonly evaluated using population-averaged measurements, yet these approaches can hide phenotypic heterogeneity that influences metabolic performance and process stability. In purple phototrophic bacteria, this limitation is particularly relevant for hydrogen-producing systems, where nitrogenase activity is tightly regulated and may vary between individual cells. The key question addressed in this work is whether nitrogenase expression is homogeneous within *Rhodospirillum rubrum* populations grown under photoheterotrophic conditions on different organic acids. To address this challenge, we developed a CRISPR-Cas9-based genetic approach to monitor nitrogenase expression at single-cell resolution. A genome-integrated fluorescent reporter was constructed by fusing the red fluorescent protein DsRed to the C-terminus of the chromosomal *nifK* gene (*nifK::DsRed*), preserving native regulatory control of the nitrogenase operon (Figure 1). Fluorescence microscopy was used to visualize nitrogenase expression within individual cells under various carbon and nitrogen regimes.

Using this system, we observed condition-dependent nitrogenase expression patterns within *Rs. rubrum* populations. Under diazotrophic growth, nitrogenase expression was homogeneous across the population, whereas under non-diazotrophic conditions, rare fluorescent cells were detected despite ammonium supplementation. These observations are supported by epifluorescence microscopy images (Figure 2), which reveal uniform expression under nitrogen-fixing conditions and the emergence of isolated nitrogenase-expressing cells under ammonium-replete growth on specific substrates, such as succinate. One possible explanation for this behavior is the presence of micro-scale heterogeneity within bacterial communities, leading to local environments where ammonium availability is reduced or transiently limiting. These results indicate that nitrogenase expression in *Rs. rubrum* is not strictly uniform across growth conditions and support the existence of metabolically specialized subpopulations. Beyond qualitative visualization, this genome-integrated reporter system offers the potential to quantify nitrogenase expression using complementary approaches such as flow cytometry, enabling high-throughput analysis of expression distributions.

The potential impact of this work lies in improving the understanding and control of purple phototrophic bacteria-based processes. By enabling visualization and quantification of functional subpopulations, this approach can inform strategies to improve PPB productivity at both metabolic and process levels, particularly for hydrogen production and biomass generation from renewable organic acids. In line with the workshop scope, this work contributes to discussions on PPB metabolism, process optimization, and the translation of single-cell phenotypes into robust and sustainable PPB-based biotechnological applications.

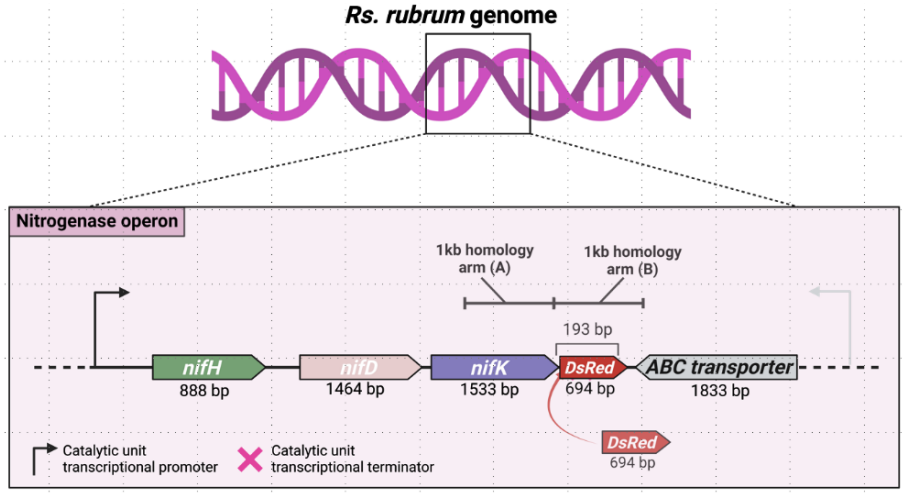

Figure 1. CRISPR-Cas9-mediated genome integration of a fluorescent reporter at the nitrogenase operon in Rhodospirillum rubrum. Schematic representation of the Rs. rubrum genome highlighting the nitrogenase operon and the strategy used to generate the nifK::DsRed reporter strain. The structural genes nifH, nifD, and nifK are shown with their respective lengths. DsRed was fused to the C-terminus of nifK via homologous recombination using two ~1 kb homology arms (A and B), resulting in a genome-integrated fluorescent reporter expressed under the control of the native nitrogenase operon promoter. The insertion preserves the endogenous transcriptional regulation of the operon and does not rely on plasmid-based expression or inducible promoters. Gene orientations, predicted transcriptional promoter and terminator sites, and neighboring genes are indicated.

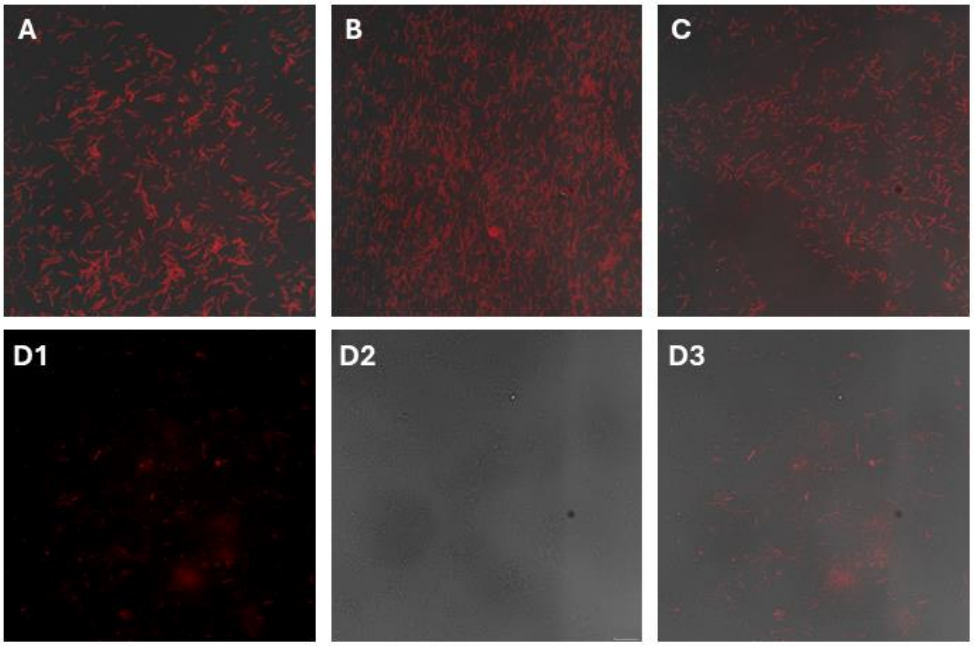

Figure 2. Evaluation of nitrogenase expression in the Rs. rubrum nifK::DsRed reporter strain under photoheterotrophic growth. Epifluorescence microscopy images (oil immersion) of Rs. rubrum nifK::DsRed cells grown under diazotrophic (A–C, 0 mM NH4Cl) or non-diazotrophic (D1–D3, 35 mM NH4Cl) conditions with 50 mM NaHCO3. Cultures were supplied with 124 mMnEqC of acetate (A), a propionate–butyrate mixture (B), or succinate (C and D1-D3). Diazotrophic images (A–C) show overlays of bright-field and red fluorescence (excitation 558 nm), indicating homogeneous nitrogenase expression. Non-diazotrophic succinate-grown pre-cultures are shown as red fluorescence only (D1), bright-field only (D2), and overlay (D3). Rare fluorescent cells are observed under ammonium-replete conditions (D1, D3), suggesting nitrogenase expression in a subset of cells despite ammonium presence.

Extraction and determination of coenzyme Q_{10}, antioxidants activities, and lipoperoxides in PNSB – a pilot study

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We developed methodological procedures for the extraction and determination of coenzyme Q_{10}, antioxidant enzymes activities (SOD, CAT, GPX), antioxidant capacity (TEAC, FRAP) and lipid peroxides (LPX) in biomass extracts of 6 PNSB strains. *$CoQ_{10\text{-}TOTAL}$ extraction*: add 0.5 mL DPBS to 2.5 mg of dry biomass, sonicate in a water bath at 40°C for 5 min, add benzoquinone (0.1 mL of 2 mg/mL H_2O stock), incubate for 10 min at 24°C, add 1 mL ethanol:isopropanol (95:5), vortex for 1 min; then add 2 mL hexane, shake for 5 min, centrifuge for 3 min at 1300 *g*, collect hexane layer - repeat another 3x; then evaporate collected layers under nitrogen at 50°C and reconstitute the extract in 0.5 mL ethanol. Determine CoQ_{10} concentration by HPLC with UV detection at 275 nm [1]. *The extract for antioxidant enzymes, TEAC, FRAP, LPX*: Add 0.25 mL DPBS to 2.5 mg biomass on ice, sonicate in a water bath at 4°C for 2x5 min, vortex for 1 min in between; centrifuge at 4°C at 10 000 *g* for 10 min; centrifuge the supernatant again, use the extract for SOD, CAT, GPX, TEAC, FRAP and LPX determination [2-7].

Table 1. Antioxidant properties and lipid peroxides in extracts from PNSB biomass

PNSB strain	$CoQ_{10\text{-}TOTAL}$ (mg/g dry mass)	SOD (U/g dry mass)	CAT (U/g dry mass)	GPX (U/g dry mass)	TEAC (µmol Trolox/g dry mass)	FRAP (µmol Trolox/g dry mass)	LPX (µmol/g dry mass)
1. Rhodopseudomonas palustris TIE1	0.901	5210	0	0	14.45	2.67	0.65
2. Rhodopseudomonas palustris 420L	1.923	525	0	0	10.40	0.49	0.05
3. Rhodospirillum Rubrum	3.163	2990	0	0	10.25	0.45	0
4. Cereibacter sphaeroides MONS	3.064	4600	0	0	8.30	0	0.11
5. Cereibacter sphaeroides (ANT)	2.557	7535	0	0	10.10	0	0.11
6. Rhodobacter Capsulatus (ANT)	2.582	8450	0	0	10.00	4.07	0.65

The highest concentration of $CoQ_{10\text{-}TOTAL}$ is in PNSB extracts No. 3 and No. 4, SOD (superoxide dismutase) is present in all extracts, but they do not contain CAT (catalase) and GPX (glutathione peroxidase). Antioxidant capacity, expressed as TEAC (Trolox equivalent antioxidant capacity) is present in all extracts and FRAP (Ferric reducing antioxidant power) is missing in samples No. 4 and No. 5. LPX (lipid peroxides) are present in all extracts except sample No. 3.

Conclusion: All PNSB biomass extracts contain antioxidants in different concentrations and activities. This pilot study confirms the highest content and activity of antioxidants in samples No. 4 *Cereibacter sphaeroides* (MONS) and No. 5 *Cereibacter sphaeroides* (ANT).

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Roadmap for evaluation of regulatory compliance of PPB-based novel food/ingredients

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Purple phototrophic bacteria (PPB) biomass, cultivated via fermentation on industrial side-streams, demands a comprehensive technical roadmap for EU pre-market authorization as a novel food under Regulation (EU) 2015/2283. Regulatory compliance ensures citizens access safer, nutritious, and environmentally responsible food choices that support wellbeing, while offering stakeholders (producers, regulators, innovators, and policymakers) a scientific basis for introducing novel bio-based ingredients with confidence in safety and acceptance.

To systematically evaluate regulatory compliance of PPB-based novel food/ingredients, an ad hoc expert group established within the Purple4Life project is developing a roadmap, specifying all required analyses and data for full EFSA submission.

The roadmap encompasses the following main technical steps, bridging data gaps for successful authorization.

Technological process mapping – This phase details the full production chain: substrate preparation (food-grade molasses/wastewater characterization for metals, mycotoxins, pesticides, microbial contaminants), bioreactor cultivation (gas mix: CO, CH_4, H_2, CO_2; parameters for biomass yield), harvesting, inactivation validation (no regrowth potential), and downstream processing (spray/freeze-drying, lipid extraction). HACCP-style tables are generated identifying stage-specific hazards/mitigations (e.g., sterilization, ultrafiltration), with validated process controls.

Strain and compositional characterization – This phase generates taxonomic identification (sequencing, strain deposition), full compositional profile (proteins, lipids, carbs, nucleic acids, carotenoids, CoQ10 quantification), and substrate residual analysis; project screens 4-6 strains.

Safety and toxicological data generation: in silico allergenicity (HRMS proteomics), in vitro cytotoxicity (Caco-2/MCS7 cells), absence of pathogenicity/toxigenicity/AMR genes, genotoxicity, acute/subchronic toxicity (in vivo if triggered), microbial safety (no E. coli/Salmonella), 90-day rodent studies, allergenicity/animal model confirmation, QPS-supporting viability assays.

Evaluation of additional dossier elements: stability/shelf-life data (storage/degradation), validated analytical methods (viable cell detection, residue quantification), exposure/intake assessments, proposed uses (food supplements, fish feed.

By piloting this roadmap for purple non-sulfur bacteria based novel food, Purple4Life will contribute to pave the way for smoother regulatory pathways.

Adhesion properties of purple bacteria and food safety

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Keywords: *surfaces, food safety, bacterial adhesion*

Surface can promote or inhibit microbial adhesion. The promotion and inhibition ability can be modified by surface coatings. In food services, safety could be increased by selecting material with low adhesion to prevent cross contamination. This type of surfaces have a strong impact in biology and food technology.

Before considering adhesion properties, surfaces need to be characterize. The characterization is made by measuring the roughness of the surface, contact angles and streaming potential. Finally, bacterial adhesion is evaluated using scanning electron microscopy and crystal violet dye methods. Fluorescence microscopy measurements is used to detect live and dead bacteria on the coated surface.

Results show that the roughness of surfaces substantially impact bacterial colonization of surfaces. In addition there is a weaker interplay with other surfaces parameters.

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Redox Balancing Mechanisms in Purple Bacteria: From Macromolecules to Metabolic Gene Expression

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Purple non-sulfur bacteria (PNSB) are metabolically versatile photoheterotrophs increasingly applied in environmental and food biotechnology. Their ability to maintain redox homeostasis, particularly under anaerobic and electron-rich conditions such as volatile fatty acid (VFA) exposure, is linked to a range of biochemical and genetic mechanisms. This study explores redox regulation in PNSB by examining macromolecular composition, isoleucine accumulation, and heterologous gene expression related to the glyoxylate shunt.

Four strains (Rs.rubrum, Rh.capsulatus, Ce.sphaeroides, and R.palustris) were cultivated under photoheterotrophic conditions with propionate and butyrate, in the presence of 50 mM sodium bicarbonate. Biomass composition was analyzed for protein, lipids, carbohydrates, nucleic acids, polyhydroxyalkanoates (PHA), and ash content. Isoleucine levels were quantified via LC-MS (ZenoTof7600) in Rs.rubrum and R.palustris grown on acetate and butyrate to assess differential metabolic responses. In parallel, aceA and aceB genes from Rh.capsulatus were cloned under the puf promoter in an mCherry plasmid and transformed into E. coli DH5α. PCR and sequencing confirmed gene insertion. The construct is now ready for conjugation into Rs.rubrum to functionally express isocitrate lyase in an ICL-deficient background.

Macromolecular profiling revealed substrate- and strain-specific shifts, demonstrating context-dependent redox strategies. Isoleucine levels differed between Rs.rubrum and R.palustris, suggesting a role in redox buffering. Successful construction of the aceAB expression vector paves the way for functional studies on glyoxylate shunt activation and its role in metabolic reprogramming. The findings advance our comprehension of the mechanisms by which PNSB influence metabolic pathways and redox states in response to fluctuations in carbon and electron balance. This understanding facilitates the development of efficient PNSB-based bioprocesses for sustainable carbon valorization within the environmental and food biotechnology sectors.

Acknowledgements:

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Optimization of Carbon Sources for Biomass and Carotenoid Production by a Newly Isolated Strain

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Purple phototrophic bacteria (PPB) exhibit remarkable metabolic versatility, allowing them to utilize diverse organic carbon substrates under anoxygenic phototrophic conditions and synthesize carotenoids with potential health benefits. (Hülsen et al., 2016). Organic acids such as acetate and succinate are generally regarded as preferential substrates for PPB growth, while sugars such as fructose can alter intracellular carbon flux distribution, energy conservation, and biomass yields through the activation of mixed metabolic pathways (Puyol et al., 2017). However, the influence of mixed carbon sources on biomass-associated carotenoid production remains insufficiently explored. The objective of this study was therefore to experimentally quantify the effects of single and mixed carbon substrates on growth kinetics, biomass formation, and carotenoid production potential in a PPB enrichment culture.

Newly isolated PPB strain was cultivated in modified Pfennig medium under strictly anoxygenic phototrophic conditions at 30 °C with continuous mixing at 150 rpm. Illumination was provided continuously using infrared (950 ± 25 nm) with intensity of 90 $\mu mol \cdot m^{2} \cdot s^{-1}$, orange (610 ± 10 nm) with intensity of 25 $\mu mol \cdot m^{2} \cdot s^{-1}$, and green (520 ± 20 nm) with intensity of 100 $\mu mol \cdot m^{2} \cdot s^{-1}$ light source. Batch cultures were grown on sodium acetate, potassium succinate, fructose, and their combinations at equivalent total carbon concentrations. Several growth models were examined for fitting the data from which modified Gompertz model was found to be the best to estimate the kinetic parameters: specific growth rate, lag phase duration, and maximum biomass concentration.

All cultures exhibited a short lag phase of approximately 7–11 h followed by exponential growth. When comparing the kinetic parameters, the best carbon source was found to be acetate plus fructose. The mixed acetate, succinate, and fructose culture did not surpass the biomass obtained with acetate plus fructose alone. The succinate plus fructose culture exhibited the lowest growth rate and the longest lag phase. A plausible explanation is metabolic incompatibility or redox imbalance when succinate and fructose are co-assimilated, potentially leading to inefficient electron distribution and delayed metabolic adaptation. It was concluded that mixed substrates enhanced growth rates but did not necessarily translate into higher final biomass.

Key words: *Purple non-sulfur bacteria (PNSB), photoheterotrophic metabolism, carbon substrates*

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Ammonium tolerance and volatile fatty acid assimilation to produce carotenoids in *Rs. rubrum*

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Anaerobic digestion (AD) is widely used to treat organic waste, generating biogas—typically composed of approximately 50% methane and 50% CO_2—and a digestate rich in ammonium and volatile fatty acids (VFAs). Interrupting AD prior to the methanogenic stage yields digestates with elevated VFA concentrations, which can be valorised using purple non-sulphur bacteria (PNSB). Due to their metabolic versatility, PNSB can produce multiple high-value compounds, including microbial proteins, polyhydroxyalkanoates (PHA), hydrogen, and carotenoids.

This work aimed to (i) assess the effects of ammonium *on Rhodospirillum rubrum* growth and VFA assimilation, (ii) characterise the carotenoids produced, and (iii) evaluate their antioxidant properties.

Rs. rubrum was cultivated in MELiSSA medium containing a synthetic digestate mixture composed of 40% acetate, 20% propionate, 15% butyrate, 5% isobutyrate, 10% valerate and 10% isovalerate. Ammonium concentrations ranged from standard (35 mM NH_4Cl) to elevated levels (70, 140, 210, 350 mM NH_4Cl). Mass spectrometry was used to monitor VFA assimilation and carotenoid profiles and for proteomic analyses.

Rs. rubrum preferentially assimilated acetic and propionic acids first, followed by (iso)butyric and valeric acids, and isovaleric acid last. Growth was unaffected at 70 mM and 140 mM NH_4Cl, whereas significant inhibition occurred at 210 mM and 350 mM NH_4Cl. Notably, propionic acid was completely consumed at all ammonium concentrations, and acetic acid was consumed almost entirely except at 350 mM NH_4Cl. In contrast, substantial amounts of (iso)butyric and (iso)valeric acids remained in the medium.

Proteomic analysis of ammonium-stressed cultures revealed candidate genes potentially required for survival under high-ammonium conditions. To investigate their functional roles, corresponding gene mutants were cultured to elucidate ammonium metabolism in *Rs. rubrum*.

Carotenoid analysis confirmed spirilloxanthin as the major pigment in stationary-phase cultures. Additional oxidation experiments were performed to identify carotenoid degradation products, and the antioxidant capacity of spirilloxanthin was assessed using oil aging assays.

Overall, *Rs. rubrum* tolerates high ammonium concentrations (up to 140 mM NH_4Cl) without growth reduction while efficiently assimilating VFAs.

Development of new and innovative harvesting and stabilization strategies for fresh purple bacteria

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The increasing demand for sustainable protein ingredients for food and feed applications is driving interest in microbial proteins produced by fermentation. These systems offer high productivity, consistent quality, and a reduced environmental footprint compared to conventional protein sources. Purple phototrophic bacteria (PPB) are of particular interest, as they combine high protein content with valuable bioactive compounds such as carotenoids, coenzyme Q10, and vitamins [1,2]. However, the use of PPB in a fresh form remains limited by challenges related to biomass harvesting, stabilization, and shelf-life extension. Conventional preservation strategies often rely on thermal treatments, which can negatively affect sensitive compounds and functional properties.

This study addresses the development of an integrated, non-thermal processing strategy to enable the production of fresh, protein-rich PPB biomass suitable for direct food and feed applications. A complete downstream processing line was investigated, including biomass harvesting, conditioning, packaging, and stabilization by high-pressure processing (HPP). Several harvesting technologies were evaluated, including centrifugation, membrane filtration, and physicochemical approaches. Their performance was assessed in terms of biomass recovery, operational efficiency, and preservation of bacterial functional properties.

Centrifugation emerged as the most robust harvesting strategy, combining high biomass recovery, operational simplicity, and limited impact on PPB functional properties. High-pressure processing (HPP) was then applied as the sole stabilization technology. While several pressure levels were explored, a validated HPP condition at 5000 bar enabled microbial load reductions of up to 6 log units under worst-case scenarios involving artificial contamination with representative Gram-positive and Gram-negative bacteria. This treatment ensured microbial stability during refrigerated storage for at least 21 days, with extended shelf life observed up to 45 days. Importantly, carotenoid retention after HPP was significantly higher than after conventional thermal treatments, demonstrating the advantage of non-thermal preservation.

Overall, this work demonstrates that coupling efficient harvesting with HPP enables the production of fresh PPB biomass with extended shelf life and preserved bioactive compounds. The proposed approach is compatible with existing industrial HPP infrastructure and supports more sustainable protein value chains by reducing thermal input, energy consumption, and processing intensity. It opens new opportunities for fresh microbial ingredient formats with clear relevance for next-generation food and feed systems.

Sis

CoQ_{10} levels in plasma in elderly people are related to their physical and cognitive capacities.

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In elderly people, functional physical capacity and body mass index are associated with the levels of CoQ_{10} found in blood plasma [1]. This relationship was stronger in women than in men. Oxidative damage found in plasma by the MDA test was inversely related to the aerobic physical capacity indicating that sedentarism was associated with higher oxidative damage in plasma. The relationship between physical capacity and plasma CoQ_{10} levels was different in young than in older people [2].

In a different study, we found that in old individuals, risk of cardiovascular disease was associated with low levels of CoQ_{10} in plasma. This relationship was clearer in women than in men [3]. Again, the levels of CoQ_{10} in plasma were associated with aerobic capacity. Further, CoQ_{10} levels were also significantly associated with muscle capacity and inversely related with sedentarism [3]. People showing risk of frailty showed lower levels of CoQ_{10} in plasma than people without risk.

We also determined the relationship of CoQ_{10} in plasma with cognitive capacity in elderly people. CoQ_{10} levels strongly and significantly correlated with the cognitive functioning, measured by the MMSE-30 score) and executive function (measured by the FAB-E score) [4]. The relationship of CoQ_{10} levels with executive function showed the highest and more significant correlation. Both tests positively correlated with the physical activity performed by the individuals throughout a week (METs) indicating that physical activity prevents cognitive and executive deterioration [5].

Altogether, our results indicate that aged people that maintain high levels of CoQ_{10} in plasma show lower oxidative damage, higher physical activity and higher cognitive and executive capacities.

In the case of the relationship of higher CoQ_{10} levels with cognitive and executive capacity we hypothesized that plasma CoQ_{10} can prevent endothelial damage and protect brain against the leaking through the brain-blood barrier. Further experiments are needed to delve into the protective effect of CoQ_{10} in elderly people.

The consumption of CoQ_{10}-rich supplements such as PPBs can help to maintain high levels of CoQ_{10} in plasma and thus, reduce endothelial damage during aging preventing by this mechanism the deterioration of cognitive and physical capacities at later years of life.

Acknowledgements:

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Theoretical and experimental assesment of the growth of *Rhodospirillum rubrum* cultivated on anaerobic digestate residues

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The anaerobic digestion is a promising way to valorise food waste into energy [1]. In addition to methane, this process produces residues that contains volatile fatty acids that may be metabolized by purple bacteria such as *Rhodospirillum rubrum* (*Rs. rubrum*) whose biomass could be further valorized. This could lead to a complete valorisation of the food waste into both energy and biomass.

Preliminary tests have shown that a wild type of *Rs. rubrum* cannot grow in a culture medium with substancial amount (over 5% vol.) of real digestate. However, successive culture with progressively increasing digestate content produced an acclimated strain able to grow into a culture medium with up to 15% vol. of digestate. The acclimated strain seems unable to grow in culture medium with higher digestate content. This could be explained either by the concentration of an unknown compound in the digestate that inhibits the bacteria growth or by the lack of available light in the culture volume. Indeed, the digestate is partially opaque (particularly to photosynthetic light), this reduces the light available for *Rs. rubrum* photoheterotrophic growth.

To assess the influence of culture medium color on *Rs. rubrum* growth, a light transfer model able to determine available light as a function of light input, culture medium color, biomass and photosynthetic pigment concentration is being developped. This model require some wavelength dependent radiative properties of the bacteria which are calculated using numerical methods [2]. The results of this study could be used in unrelated work to optimize the input light spectrum and maximise the light power to biomass ratio.

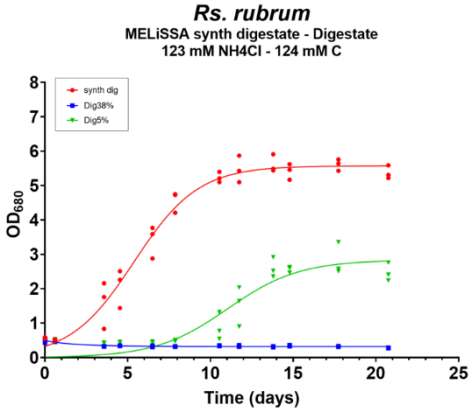

Figure 1. Growth of *Rs. Rubrum* in culture medium with synthetic (transparent) digestate, 5% vol. and 38% vol. of real digestate (partially opaque).

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Impact of light regimes and carbon loading on the growth of *Rhodospirillum rubrum* cultivated on sugar beet molasses for food and feed applications

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Introduction

The need for food production is expected to increase by approximately 1.4% per year over the next decade due to population growth and rising incomes in several countries [1]. This growth may lead to an increase in accumulation of agro-industrial by-products. Among these, sugar beet molasses, which is a major co-product of the sugar industry, constitute a carbon-rich substrate for microbial valorization [2]. In this context, a circular economic approach appears promising to limit the associated environmental impacts. However, the use of molasses by purple non-sulfur bacteria mainly focused on processes considered as a downcycling such as photo-fermentation for biohydrogen production. The development of upcycling strategies could offer a promising approach by using molasses as a substrate for *Rhodospirillum rubrum* for food and feed applications. One of the main remaining technical challenges is curbing the access of *Rs. rubrum* to the market is its high production cost predominantly linked to culture lighting and the lack of LEDs optimization specific to purple non sulfur bacteria requirement. Moreover, at the culture level, light-limited conditions, caused by insufficient light penetration in the system, can further decrease the biomass productivity caused by a metabolic shift of the culture from photoheterotrophy to undesired fermentation metabolism. This study will evaluate different light sources to identify those that maximize photosynthetic growth while minimizing undesired fermentation aiming to improve overall efficiency of molasses upcycling into microbial biomass for human and animal feed applications.

Materials and Methods

Using a glucose-acclimated *Rhodospirillum rubrum* strain (*Rs. rubrum* MG), two complementary experiments will be conducted to optimize growth conditions for biomass productivity. In the first experiment, bacterial cultures will be grown in 200 mL plastic bags containing molasses under three light regimes: (i) halogen light (10 W, 3000 K, warm white), (ii) a combination of Vege Red® LEDs (25.9 W, 3000 K) and Vege Red® infrared LEDs (25.9 W, 840–850 nm), and (iii) red light using Vege Red® LEDs (Far Red 750 nm, Far Red 730-740nmand Cherry Red 655–665 nm, 14.4 W each). Light energy input, biomass yield, and production costs will be evaluated, while fermentation profiles, pigment composition, and proteomic analyses will be performed to assess the functional properties of the biomass for potential marketable products.

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Making PNSB lipid signatures comparable: a practical workflow to access membrane fatty acids for food/feed positioning

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A recurring bottleneck in purple non-sulfur bacteria (PNSB) biomass valorization is not the absence of promising bioactive lipids, but the difficulty of generating comparable, decision-grade lipid evidence across batches, strains, and cultivation conditions. This challenge is inherently biological: fatty acids are largely embedded in membrane and intracytoplasmic membrane architectures. Their apparent profiles shift with physiology, making lipid "fingerprints" highly sensitive to biomass processing and to how analytical ambiguity is handled. Using *Rhodospirillum rubrum* as a reference PNSB system, this work focuses on fatty-acid signatures where isomeric ambiguity matters for biological interpretation and downstream positioning (e.g., vaccenic-acid isomers).

Within the PurpleHealth project, a recovery-aware high-resolution LC–MS workflow is under design to progressively access the membrane-associated fatty-acid pool and stabilize fingerprints across analytical sequences. The workflow combines (i) organic extraction, (ii) alkaline hydrolysis to release esterified fatty acids from complex lipid structures, and (iii) 3-nitrophenylhydrazine (3-NPH) derivatization to improve detectability and chromatographic behavior. Measurements are performed on a high-resolution LC–MS platform (ZenoTOF 7600), leveraging accurate mass and targeted fragmentation to increase structural confidence and to discriminate biologically meaningful unsaturated fatty acids, including sensitive isomeric pairs such as vaccenic cis/trans. This is particularly critical for PNSB datasets, where subtle shifts in isomer composition can reflect distinct metabolic states and influence how lipid evidence is interpreted for downstream positioning.

A key contribution is turning quality control routine into a PNSB-specific comparability framework. Multi-level parameters and dual controls are structured to separate ubiquitous background fatty acids from biomass-derived features, to monitor drift and sequence-dependent instability. The workflow is explicitly built to separate biological signals from artefacts, and to identify which analytical descriptors remain stable under realistic variability.

Overall, this workflow aims to move PNSB lipid evidence from presence/absence toward ingredient-relevant characterization that can support food/feed positioning and future health-oriented investigation. Thus, feedback from the PNSB community is appreciated, on the most decision-relevant analytical descriptors for translating PNSB lipidomics into actionable food/feed evidence, including which descriptors best capture robustness across strains, cultivation regimes, and batch variability.

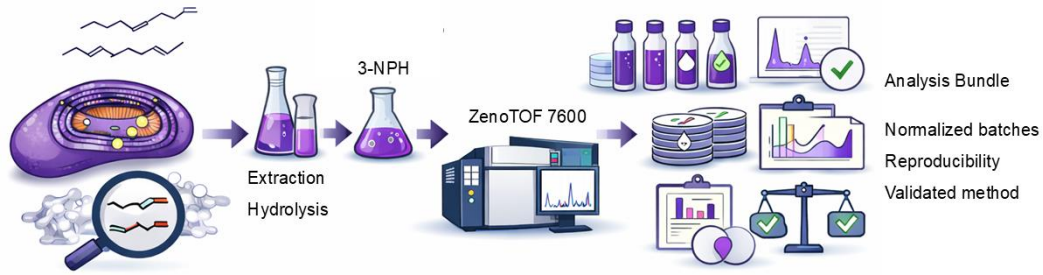

Figure 1. Workflow of PNSB lipidomics

Selection and cultivation of Purple Sulphur Bacteria for biogas desulfurization

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This work aims to select and cultivate a consortium of Purple Sulfur Bacteria (PSB) for biogas desulfurization and valorization. In fact, biogas typically contains H_2S that has to be removed before its direct employment for the cogeneration of heat and power or its upgrading to a renewable CH_4 stream; the metabolic activity of PBS consortia can couple the biological removal of H_2S to the sustainable production of elemental sulfur (S^0)-rich biomass that can be applied as a slow-release fertilizer agent, and/or cell proteins.

A digestate from the anaerobic digestion of sewage sludge was used as the process inoculum and enriched in PSB by using simple selective pressure allowing fully phototrophic technologies (by applying cheap UV-VIS filters to sunlight spectrum). Experiments were carried out under a semi-continuous regime in 1 L Pyrex bottles with screw caps with a working volume of 0.6 L at 29.0 ± 1.5 °C under N_2 atmosphere; bicarbonate was used as the sole electron acceptor and sulfide as the electron donor. The digestate was used as an effective source of nutrients without micronutrients and vitamins addition, in the perspective of operating in an upscaling process where the addition of extra elements means supplementary costs; sulfide, phosphate and nitrate were added to reach the concentrations reported for medium. pH was adjusted to 7.3 and flasks were placed on a shaker at 150 rpm under 24 h light by a halogen 500 W lamp at a light intensity 50 W m^{-2}. To prevent the competition of microalgae and green sulfur bacteria, the bottles were covered with UV-VIS absorbing foil (ND 1.2 299, Transformation Tubes, UK) (Fig. 1). After multi-cycles feeding of substrates, steady-state conditions were reached, and the concentration of PSB-enriched biomass was about 3.8 g TSS L^{-1}. A multi-step reaction occurred, producing S^0 as an intermediate and sulfate as the final oxidation product, by an overall complete sulfide removal. Maximum S^{2-} removal rate of 3.5 mg S^{2-} L^{-1} h^{-1} was obtained by applying multiple pulses of sulfide feeding.

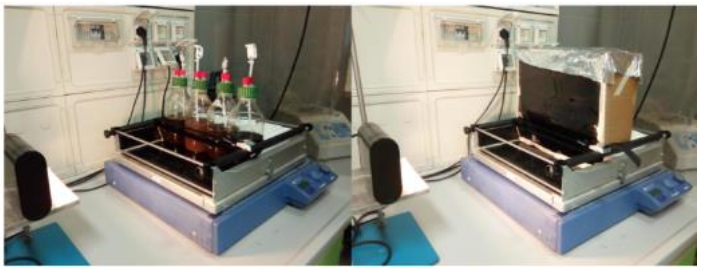

Figure 1. Experimental set-up.

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Selection and cultivation of Purple Non-Sulphur Bacteria for the post-treatment of anaerobic effluents

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This work aims to select and cultivate a consortium of Purple Non-Sulfur Bacteria (PNSB) for the post-treatment and valorization of short chain volatile fatty acids (VFA)-rich effluents originated by the anaerobic acidogenic fermentation of organic residues generated by the fish processing industry. These streams are characterized by high organic load and significant concentrations of VFAs, representing both an environmental challenge and an opportunity for biotechnological valorization.

Two sludges were collected from the Navile channel of Bologna (Italy) in proximity of the outlet of the local wastewater treatment plant (WWTP), and from the denitrification vessel of the WWTP of Parma, and centrifuged (10000 rpm for 10 min at 4°C). The resulting pellets were singularly used as inoculum (10% v/v) to carry out duplicate tests conducted in N_2-insufflated and closed 500 mL-flasks. The cultures were inoculated in a modified DSMZ medium 28 without H_2S and amended with molybdate; however, no vitamins were added. Concentrated acetic, propionic and butyric acids, in the ratio of 3:1:1, respectively, were added to get an initial overall concentration of 0.5 g/L. Bottles were incubated at room temperature under continuous illumination shielded by infrared transmission filters. After one month of preliminary batch process, the cultures were fed in semi-continuous mode for about two months, by replacing part of the exhausted broth with fresh amended medium, with an overall HRT of 40 days. Consecutive semi-continuous experiments were carried out by inoculating further bottles with spent broths (10% v/v) and the same fresh amended medium until a red/brown color was observed. On the bases of OD600 nm (1,980), pH (8,49), acids consumption and color parameters, one of the bottles obtained by initially using the "Navile" sludge was selected as the inoculum for scaling-up the culture growth in a self-designed and constructed 3 L-photobioreactor (Fig.1A). The reactor was operated under same strategy and conditions by which screening experiments were carried out for 70 days, during which an effective VFAs consumption, as well as a noticeable increase in OD600 nm (from 0,345 to 1,800) and pH (from 6,92 to 8,97), were observed. Bacterial growth was also monitored by checking the color and biofilm growth (Fig. 1B).

Beyond evaluating the capability of the PNSB consortium to grow on acid rich effluents, the recovery and characterization of the resulting biomass represent an additional research perspective. PNSB cells will be harvested, lyophilized, and subjected to extraction procedures in order to assess their potential bioactivities. In particular, the extracts will be evaluated for antimicrobial, lipid-reducing, and antidiabetic properties.

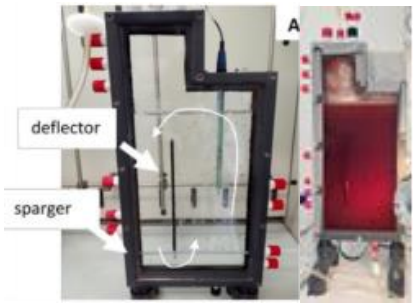

Figure 1. Photobioreactor set up (A) and operated (B) for the growth of the selected PNSB consortium.

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