2nd PHOTOREFINERIES CONFERENCE

BOOK of Abstracts

2nd International Conference on Novel Photorefineries for Resource Recovery

2nd - 4th September 2026
Faculty of Tehnology Novi Sad
University of Novi Sad, Serbia



European COST Action
CA21146 - Fundamentals and applications of purple bacteria biotechnology for resource recovery from waste (PURPLEGAIN)

cost EUROPEAN COOPERATION IN SCIENCE & TECHNOLOGY

2nd Photorefineries Conference

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GENERAL INFORMATION

The PHOTOREFINERIES CONFERENCE is an event initiated within the framework of the European COST Action PURPLEGAIN (CA21146), a project funded by the European Cooperation in Science and Technology (COST) programme.

The PURPLEGAIN COST Action aims to establish a European network of scientists and experts, particularly those focusing on the fundamentals and applications of purple phototrophic bacteria (PPB) for resource recovery. This network fosters information exchange and facilitates technology and knowledge transfer between academia and industry in this specific biotechnology field. The Action addresses resource recovery from wastewater and organic waste in both open and closed environments, through single-step as well as integrated processes. Finally, PURPLEGAIN seeks to contribute to the advancement of a sustainable circular bioeconomy at the global level.

Building on the vision of PURPLEGAIN, the PHOTOREFINERIES CONFERENCE expands the scope beyond the anoxygenic photosynthesis and works on the development of photo-driven processes in sustainable biorefineries, bringing together scientists, engineers, and experts working in the fields of microbial fundamentals, photocatalysis, microbial photosynthesis, artificial photosynthesis, photo(bio)reactor development, mathematical modeling, and biorefinery processes. The focus of the conference is on photo-activated processes for sustainable production of commodities using light as the common encounter point. Special attention is given to microbial and chemical processes for the conversion of residual streams into high value-added products such as polyhydroxyalkanoates, microbial protein, hydrogen, carotenoids, terpenoids, corrinoids, organic acids, coenzyme Q10, and 5-aminolevulinic acid, along with biomass applications for energy and fertilizers.

Following the success of the 1st PHOTOREFINERIES CONFERENCE, held at the University of Valladolid, Spain, in 2024 and attended by over 170 participants from 26 countries, the 2nd edition will be hosted as a 3-day event from 2nd to 4th September 2026 at the University of Novi Sad, Serbia.

By fostering dialogue across disciplines and expertise, the 2nd PHOTOREFINERIES CONFERENCE provides a platform for efficient discussions on the latest scientific discoveries, industrial applications, and future perspectives - while accelerating the development of sustainable pathways and innovative solutions based on light-driven processes in photo-refineries.



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https://purplegain.eu/

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SCIENTIFIC TOPICS

- PHOTOREFINERIES BASED ON PURPLE PHOTOTROPHIC BACTERIA (PPB)
- BIOREFINERIES BASED ON MICROALGAE OR CYANOBACTERIA
- PHOTOSYNTHETIC BIOTECHNOLOGY FOR WATER AND AIR TREATMENT
- TECHNOLOGIES FOR POST-TREATMENT, EXTRACTION AND PURIFICATION OF BIOMASS AND BIOPROCESS RESIDUAL STREAMS
- ADVANCED CONTROL, MODELLING AND ASSESSMENT OF PHOTOACTIVATED PROCESSES
- PHOTOCATALYTIC PROCESSES AND ARTIFICIAL PHOTOSYNTHESIS
- MICROBIAL ECOLOGY & PHYSIOLOGY OF PPB AND OTHER PHOTOTROPHS
- TECHNO-ECONOMIC AND LIFE CYCLE ASSESSMENT OF PROCESSESS INVOLVING PHOTO-ACTIVATED PROCESSES IN PHOTOREFINERIES
- PHOTOREACTOR DESING: CHALLENGES AND FUTURE DIRECTIONS

Social networks for European COST Action CA21146 - Fundamentals and applications of purple bacteria biotechnology for resource recovery from waste (PURPLEGAIN)

KEYNOTE, PLENARY

ANPHORA®: A UNIQUE PURPLE PHOTOTROPHIC BACTERIA-BASED PLATFORM FOR LOW-ENERGY URBAN WASTEWATER TREATMENT AND RESOURCE RECOVERY

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Purple phototrophic bacteria (PPB)-based wastewater platform offers an alternative to conventional energy-intensive treatment by coupling organic matter and nutrient removal with biomass for agricultural use. ANPHORA® is a PPB-driven platform implemented in open anaerobic photobioreactors for urban wastewater treatment. The technology was developed and validated in DEEP PURPLE and further consolidated through the WATERSENS and POST PURPLE projects.

The technology has been validated at two demonstration sites in Spain. The Linares WWTP plant treats 350 m3/day (approximately 1,500 population equivalent) and has operated since 2022 as the main reference facility for long-term optimisation. A second demonstrator at Rincón de Caya WWTP (Badajoz), designed for 150 m3/day (approximately 550 population equivalent) and operating since 2025, represents the replication step. Under hydraulic retention times below 48 h, the process achieved effluent concentrations below 40 mg/L total COD, 23 mg/L BOD5, 15 mg/L total suspended solids and 0.7 mg/L total phosphorus. The demonstrations also confirmed the low-energy character of the process, with electrical consumption below 0.2 kWh/m^3 and on-site photovoltaic supply.

Current follow-up activities focus on techno-economic optimisation of the ANPHORA® platform. POST PURPLE supports the use of low-cost luminescent filter materials in greenhouse-type covers for an expected 21% CAPEX reduction, while WATERSENS develops advanced monitoring and supervisory control strategies to reduce specific energy consumption. Overall, the results obtained at Linares and Badajoz indicate that PPB-based photobiorefineries can combine compliant effluent quality, low electrical demand and improved techno-economic performance, supporting their scalability as circular solutions for municipal wastewater treatment.

Keywords: ANPHORA®, purple phototrophic bacteria, urban wastewater treatment.

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HARNESSING PURPLE PHOTOTROPHIC BACTERIA TO SHAPE THE NEW FOOD LANDSCAPE: FROM SPACE INSPIRED CONCEPTS TO SMART, CIRCULAR NUTRITION AND BIOECONOMY

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Purple phototrophic bacteria (PPB) offer a unique opportunity to redesign the future food ecosystem thanks to their remarkable metabolic versatility and their capacity to upgrade agro industrial side streams into high quality, nutrient dense biomass. Their ability to assimilate diverse carbon sources—including whey, molasses and volatile fatty acids—positions them as powerful microbial platforms for circular bioprocessing.

The concept of consuming microbial biomass such as purple bacteria traces back to ESA's MELiSSA program, which pioneered closed loop, regenerative life support systems capable of converting organic waste into edible material for long duration space missions. This visionary framework laid the foundation for considering PPB as food ingredients. Building on this legacy, our UMONS team now develops low cost yet smart PPB production strategies that translate space derived bioprocess principles into economically viable terrestrial applications.

Within the FoodWal PROTEBOOST project, PPB are being transformed into protein rich food ingredients through innovations spanning harvesting, preservation and formulation of food grade biomass. In parallel, the Horizon Europe Purple4Life initiative advances scalable PPB cultivation for food and feed applications and validates their safety through allergenicity and cytotoxicity assessments. Just create Spin-Off company PurpleTech is now going the extra mile to industrialization by improving productivity to reach economic viability. Health promoting effects demonstration in humans, but also in aquaculture, is also central to our activities.

This presentation will summarize how we integrate space inspired concepts, metabolic knowledge and smart tech industrialisation—driven by our spinoff Purpletech—to establish a PPB based circular economy and position these bacteria as transformative actors within the emerging sustainable food landscape.

Keywords: Circular economy, health-promoting ingredient, PPB,

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THE QUEST FOR VALUE IN PNSB BIOTECHNOLOGY: FROM DIRECT RECOVERY TO HIGH-VALUE FROM GAS

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Purple non-sulfur bacteria (PNSB) owe their biotechnological appeal to an unusually versatile metabolism and extremely high product yield. For over a decade, the dominant paradigm for their application has been direct recovery from waste streams: enriched PNSB mixed cultures grown photoheterotrophically on organics present in wastewater or liquid fermentation effluents, yielding biomass for use as microbial protein (MP) for feed and biofertilizer, alongside polyhydroxyalkanoates, pigments, and H_2. This approach has matured considerably, supported by numerous studies, pilot and demonstration scale data, and mechanistic models capable of describing outdoor, light- and temperature-dependent growth in both photobioreactors and raceways. However, this approach remains intrinsically tied to the variability, contamination risk, and limited added value of biomass grown directly in waste-derived feedstocks.

A more recent line of work proposes feeding PNSB waste-derived gaseous electron donors and nutrients: H_2 and CO_2 for autotrophic growth, and N_2 for diazotrophic growth. This shift unlocks three decisive advantages over the "direct recovery" paradigm: substrate sterility, which removes pathogen-transfer concerns from the resulting biomass, consistent biomass properties over time, and substantially higher product value, since the resulting biomass could qualify as novel-food-grade MP rather than a recovered by-product. Recent results from gas-fed PNSB cultures show protein yields and specific uptake rates among the highest reported for any gas-fermenting microorganism, exceeding hydrogen-oxidizing bacteria on H_2/CO_2, and outperforming other diazotrophs on N_2, while delivering complete dietary amino-acid profiles for human nutrition.

This talk traces that trajectory, from the early results and techno-economic drivers identified for waste-fed PPB systems to the kinetics, modelling, and scale-up challenges now defining gas-to-protein PNSB processes, and closes with a perspective on what it will take to move these systems from batch proof-of-concept to continuous, decarbonized, industrially relevant processes.

Keywords: Purple non-sulfur bacteria; resource recovery; microbial protein; photoheterotrophy; photoautotrophy; mechanistic modelling

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PURPLEGAIN: CATALYSING PURPLE PHOTOTROPHIC BIOTECHNOLOGY IN EUROPE

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Abstract

Purple phototrophic bacteria have attracted scientific and technological interest for more than six decades due to their exceptional metabolic versatility and their ability to convert organic residues, inorganic compounds, and light energy into valuable products. Since the first technological studies in the 1960s, research has progressively moved from fundamental physiology to applications in wastewater treatment, resource recovery, biohydrogen production, microbial protein, bioplastics, pigments, and other high-value compounds. In Europe, this development has accelerated through the emergence of specialized research groups, pilot-scale demonstrations and collaborative projects focused on integrating purple bacteria into circular bioeconomy and biorefinery concepts.

Despite this progress, the European community remained fragmented across countries, disciplines and application areas. PurpleGain was created within this context to connect researchers, technology developers, companies and other stakeholders working on purple bacteria and related phototrophic processes. The Action aimed to strengthen scientific cooperation, support knowledge exchange, promote researcher mobility and increase the visibility and technological maturity of the field.

This presentation will first provide a historical overview of the technological development of purple bacteria since the 1960s, followed by a review of the emergence and consolidation of purple-bacteria biotechnology in Europe. It will then describe the scientific and collaborative environment in which PurpleGain was established.

The main part of the presentation will review the evolution of the COST Action year by year, highlighting its meetings, thematic workshops, training schools, Purple Bubbles and international conferences. Attention will be given to Short-Term Scientific Missions, fellowships and other mobility instruments that enabled researchers, especially young scientists, to access complementary expertise, develop new methods and establish long-term collaborations.

Finally, the presentation will discuss the scientific, educational, and networking impact of PurpleGain, its role in consolidating an international purple-bacteria community, and the opportunities to maintain this momentum beyond the lifetime of the Action.

Keywords: Purple phototrophic bacteria; biotechnology; photobiorefineries; COST Action; scientific networking.

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FACILITATING HARVESTING OF MICROALGAE THROUGH FLOCCULATION

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Harvesting of microalgae using either centrifugation or filtration remains challenging because of the small cell size and low biomass concentration. Flocculation can facilitate harvesting by forming larger aggregates that are more readily separated from the culture medium via filtration or centrifugation. However, a key drawback of flocculation is biomass contamination with chemicals with chemicals that can interfere with downstream valorisation. This limitation can be reduced by using bio-based flocculants such as chitosan. Alternatively, it can be avoided by employing flocculation systems in which the flocculant can be removed after harvesting, for example through magnesium hydroxide precipitation or pH-reversible flocculants. Flocculation can be combined with gravity sedimentation but this is a slow proces that produces a biomass concentrate with a high water content. Therefore, we explore dissolved air flotation as an alternative to gravity sedimentation.

GROWING PHOTOTROPHS IN THE WORLD'S SUNNIEST CONTINENT: THE PPB JOURNEY AT UQ

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This abstract provides an Australian perspective of the initial development of practical technologies utilizing mixed culture phototrophic bacteria. The original focus was on domestic wastewater treatment, and inspired by Perry McCarty (anaerobic) and Willy Verstraete's ideas to fully achieve resource recovery by focusing wastewater treatment on complete resource recovery, rather than incremental changes to activated sludge concepts. Tim Huelsen's original PhD work in 2013 identified the potential to completely capture resources, in a variety of configurations, including high salinity and low temperature environments. However, there remained major challenges for domestic wastewater, including high C:N ratios in the captured biomass, and high anaerobic recalcitrance, surprising given the rapid growth rates and short sludge ages. Some of these issues have been resolved by other researchers, including Daniel Puyol, who established his own research group, and PURPLEGAIN following a research fellowship at UQ. Our focus subsequently shifted to closing resource cycles in agri-industrial research, including demonstrating industrial scale in animal production and processing, a large scale fish feed trial, which demonstrated commercial viability for PPB as a fish feed substitute, and development of granular options for PPB growth, which simplifies animal feed production. European research has demonstrated their broader utility as feed and fertilizer products which have amplified effects beyond mineral fertilizer. The last 10 years have seen rapidly diversifying applications for these highly versatile organisms, with them now representing a diversified platform, rather than collection of applications. This has expanded to mixed culture gas biotechnology, and generation of high-value products, including pigments, vitamins, colors, biopolymers, stronger industry participation, and a re-evaluation of how the foundational pure culture science underpinning this work applies to mixed culture, open environment biotechnology platforms. PPB are a classic example where broad metabolic capability implies broad applications (cf, Gabriel Capson Tojo and Daniel Puyol), and by that metric, we still have more applications for these fascinating organisms.

Keywords: Review, model, domestic wastewater

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TOWARDS DIGITAL TWINS IN MICROALGAE PRODUCTION

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The urge for food security and sustainability has advanced the field of microalgal biotechnology. Microalgae are microorganisms able to grow using (sun)light, fertilizers, sugars, CO_2, and seawater. They have high potential as a feedstock for food, feed, energy, and chemicals. Microalgae grow faster and have higher areal productivity than plant crops, without competing for agricultural land, and with 100% efficient uptake of fertilizers, if the process is well designed and controlled. Compared with bacterial, fungal, and yeast single-cell protein production based on hydrogen or sugar, microalgae show higher land-use efficiency. New insights are given into the potential of microalgae to replace soy protein, fish oil, and palm oil, and to be used as cell factories in modern industrial biotechnology to produce designer feed, recombinant proteins, biopharmaceuticals, and vaccines.
In addition, the importance and steps towards automation and digitalization of microalagae production at AlgaePARC will be presented

Keywords: Keyword 1, Keyword 2, Keyword 3 etc. Microalgae, protein, oils, scale-up, biotechnology

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BEYOND THE SURFACE: A PERSONAL JOURNEY INTO SUSTAINABLE CATALYSIS

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A Heterogeneous catalysis based on readily available, well-defined nanomaterials, single-atom or metal-free catalysts is attracting growing interest, driven by the increasing pressure on industry to adopt more sustainable chemical production schemes. In the energy sector, nanostructured catalytic materials have demonstrated remarkable potential for the efficient conversion of biomass [1–2] as well as for the production of hydrogen and liquid hydrogen carriers [3–5], organic synthesis [6] and as well as in challenging CO_2 to ethylene photocatalytic conversion [7]. These emerging catalytic systems are expected to move beyond fundamental research and stimulate industrial interest in the development of green organic synthesis, renewable energy conversion, and pollution prevention and control - key challenges of the 21st century and the central focus of this presentation.

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MEDIATED PHOTOELECTROCHEMICAL SYSTEMS AS A PROXY FOR ARTIFICIAL PHOTOSYNTHETIC SCHEMES: A NEW PARADIGM FOR PHOTOREFINERIES

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Natural photosynthesis is the ultimate photorefinery: it captures solar energy, separates photogenerated charges, uses molecular redox carriers to couple spatially separated reactions, and converts simple feedstocks into energy-rich and value-added products under mild conditions. Yet, its extraordinary biological integration also imposes a level of molecular and structural complexity that is difficult to reproduce in fully synthetic systems. This keynote proposes mediated photoelectrochemical systems as a complementary proxy for artificial photosynthetic schemes and introduces the MeTaPEC (Mediated Tandem PhotoElectroChemical) concept as a new paradigm for light-driven photorefineries.

In MeTaPEC, the functions that are integrated within the photosynthetic apparatus are deliberately decoupled and re-engineered using semiconductor photoelectrodes, electrochemical interfaces and reversible redox mediators. The mediator plays a role conceptually analogous to the mobile electron-transfer components of natural photosynthesis, but with a crucial difference: its redox potential, concentration, transport, and regeneration can be independently designed. This creates a modular architecture in which mediated photoelectrochemical oxidation (MPEO) and mediated photoelectrochemical reduction (MPER) can be optimized separately and subsequently coupled in a tandem configuration.

Herein, I will discuss the conceptual foundations of MeTaPEC, its analogy with the architecture of natural photosynthesis, and the scientific challenges that must be addressed to transform mediated photoelectrochemistry into a genuine artificial-photosynthetic platform. The central proposition is that the future of photorefineries may not lie in mimicking nature molecule by molecule, but in abstracting and re-engineering the functional principles that make photosynthesis so powerful.

Keywords: artificial photosynthesis, mediated photoelectrochemistry, photorefineries, redox mediators, solar fuels

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Section 1.
PHOTOREFINERIES BASED ON PURPLE PHOTOTROPHIC BACTERIA (PPB)

ENHANCING PHA PRODUCTION IN PPB VIA pH-INDUCED STRESS BEYOND NUTRIENT LIMITATION

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Purple Phototrophic Bacteria (PPB) are proposed as a versatile resource-recovery platform due to their ability to thrive on diverse organic substrates under anaerobic, illuminated conditions. Among bioproducts, polyhydroxyalkanoates (PHAs) have gained attention as biodegradable alternatives to petroleum-based plastics. However, achieving high PHA accumulation in PPB remains challenging due to their metabolic flexibility, particularly their ability to fix nitrogen, which often prevents carbon storage. This study evaluates the effects of environmental stressors (nitrogen availability and pH) on PHA accumulation capacity in a PPB-enriched mixed culture.

Batch experiments (2 L) were performed over 72 h cycles, at 25 ºC, testing seven conditions. A control (B0), fed 520 mg $COD_{acetate}$/L and 106 mg NH_4^+-N/L, was compared against pulse-feeding strategies: i) C-N pulses (B1, B2) using B0-media at 0 h, ii) C-only pulses (B3, B4) using B0-media at 0 h, iii) C-only pulses (B5, B6) using B0-media without N at 0 h. Each pulse supplied 520 mg COD/L. Each strategy was evaluated at two pH levels: acidic pulses (pH 3.5, B1, B3, B5) and pH-adjusted pulses (6.5, B2, B4).

Nitrogen limitation remained a primary driver of PHA synthesis, with PHA yields up to 30% dw in B5. However, pH emerged as a significant metabolic stressor for PHA accumulation to maintain cellular balance. Acidic pulses consistently enhanced PHA production across all tested conditions. In nitrogen-limited tests, accumulation dropped from 30% (B5) to 12% (B6) when the pH was adjusted to 6.5. PHA accumulation was barely detected (1% dw), but with the same C-N ratio (B1 and B2), it increased to approximately 10% in B1. Interestingly, even in the presence of nitrogen, acidic pulses triggered accumulation of up to 19% (B3). These results demonstrate that low pH acts as an effective metabolic stressor, redirecting PPB metabolism towards intracellular carbon storage even in nitrogen-rich environments. This finding is particularly relevant for industrial applications, as many organic waste streams contain high nitrogen concentrations that typically hinder PHA accumulation. This expands the niche application of PPB-based photorefineries, offering a robust alternative to conventional aerobic cultures by leveraging environmental stressors beyond conventional nutrient starvation.

Keywords: metabolic stress, mixed microbial culture; polyhydroxyalkanoates; PPB; resource recovery.

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VALORIZATION OF SLAUGHTERHOUSE WASTEWATER FOR PROTEIN RECOVERY VIA PURPLE PHOTOTROPHIC BACTERIA: COMPARISON OF RAW AND FERMENTED SUBSTRATES

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Slaughterhouse wastewater (SWW) is characterized by high concentrations of organic matter and nutrients, offering considerable potential for resource recovery within the framework of the circular bioeconomy and zero-waste strategies. In this context, and as consistently demonstrated in the literature, acidogenic fermentation converts complex organic matter into volatile fatty acids (VFAs), which are the preferred carbon source for purple phototrophic bacteria (PPBs), enabling efficient photoheterotrophic growth and protein-rich biomass production. This study aimed to evaluate biomass growth, protein productivity, and nutrient removal of a PPBs consortium by comparing the performance of raw (RSWW) and fermented (FSWW) substrates. Two SBR photobioreactors were operated in parallel under identical conditions (3.5 kg COD kg^{-1} TSS d^{-1}, 24-h cycle) under infrared light irradiation, for 85 days. Fermentation produced a VFA mixture up to 1651.6 ± 684.6 mg L^{-1}, predominantly composed of propionate (469.2 ± 195.5 mg L^{-1}), acetate (353.5 ± 121.4 mg L^{-1}), and butyrate (310.4 ± 145.6 mg L^{-1}), along with branched-chain acids such as isobutyrate and isovalerate. The system fed with FSWW demonstrated a substantial improvement in performance, achieving an average biomass productivity of 292.7 ± 102.4 mg VSS L^{-1} d^{-1}, approximately 2.4 times higher than that obtained with RSWW (120.0 ± 221.5 mg VSS L^{-1} d^{-1}). Biomass growth over a 24-hour cycle reached 14.5% with FSWW, compared to 5% with RSWW, indicating greater substrate bioavailability and metabolic efficiency. Although the protein content of the biomass was similar in both systems (32.8 ± 6.4% for FSWW and 34.6 ± 8.9% for RSWW), the fermented substrate achieved higher biomass productivity and lower variability, reflecting greater metabolic efficiency and process stability. Additionally, nutrient removal efficiencies were significantly higher in the FSWW system, achieving 22% removal of total Kjeldahl nitrogen and 43% of phosphate, compared to 5% and 33%, respectively, in the RSWW system. Overall, the results demonstrate that acidogenic fermentation enhances the conversion of complex organic matter into VFAs, optimizing PPB performance and enabling more stable and efficient protein recovery.

Keywords: purple phototrophic bacteria, slaughterhouse wastewater, volatile fatty acids, protein recovery, circular bioeconomy

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WINERY WASTEWATER TREATMENT AND BIOPRODUCT GENERATION USING PURPLE PHOTOTROPHIC BACTERIA IN AN OPEN RACEWAY SYSTEM

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Purple phototrophic bacteria (PPB) are metabolically versatile microorganisms capable of growing under photoheterotrophic conditions while converting organic substrates into value-added products. Because of these characteristics, PPB have gained attention within the circular economy as a promising alternative for the treatment and valorization of agro-industrial wastewaters. In this study, the feasibility of treating winery wastewater using a PPB-enriched culture in an open raceway reactor was evaluated, focusing on organic matter removal, microbial community stability, and the production of bioproducts, including polyhydroxyalkanoates (PHA), pigments, biomass, and 5-aminolevulinic acid (5-ALA). The system was inoculated with a culture enriched in PPB and operated under reductive photoheterotrophic conditions (ORP lower than -200 mV) in an open raceway reactor. The reactor was initially operated in sequencing batch reactor (SBR) mode to promote selective enrichment and stabilization of the microbial community, and then switched to continuous mode. During this stage, hydraulic residence times of 4,6 and 8 days were applied while treating white-wine wastewater with an initial chemical oxygen demand (COD) concentration of approximately 3.9 g COD/L. Under these conditions, the system achieved high treatment efficiencies, reducing COD to approximately 0.19 g COD/L. Biomass concentrations increased progressively, reaching up to 5.5 g volatile suspended solids (VSS)/L, demonstrating the open system's ability to sustain a dense, stable microbial consortium. In addition to wastewater treatment, the enriched biomass produced valuable metabolites. PHA accumulated in the biomass at levels close to 20%, 12 mg bacteriochlorophyll/gVSS, and concentrations of 8 µmol 5-ALA /L were detected in the culture medium. These results highlight the potential of PPB-based systems to simultaneously remove organic matter and generate compounds of industrial interest. Microbial community analysis confirmed a successful enrichment of PPB in the reactor. The genus *Rhodopseudomonas* dominated the microbial community, maintaining relative abundances above 95% for approximately 90 days of operation. Finally, during continuous operation at an organic loading rate of up to 2 g COD/L·d, the reactor achieved higher absolute organic matter removal rates while maintaining stable biomass production and bioproduct formation, demonstrating the robustness of the open raceway system.

Keywords: PPB, raceway reactor, COD removal

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DEGRADATION OF SOFTWOOD LIGNOSULFONATE BY PURPLE BACTERIUM *Rhodopseudomonas palustris*

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Pulp and paper industries generate large amounts of technical lignins. Due to their intrinsic structural complexity, further enhanced by the harsh process conditions utilized for its isolation, technical lignins are mainly burned to produce heat, contributing to greenhouse gas emissions. A more sustainable alternative for their treatment could involve ligninolytic microorganisms and their enzymes.
In this framework, the present study investigates, at a macroscopic level, the ability of *Rhodopseudomonas palustris*, a purple non-sulfur bacterium characterized by noticeable metabolic versatility, to grow using softwood lignosulfonate (SLS) as sole carbon source. More in detail, to lower the content of carbohydrate impurities in SLS, this last was subjected to methanol-aided fraction, which allowed for the isolation of a purer methanol-insoluble cut (MISLS), prior to the incubation with *Rhodopseudomonas palustris*.
A semicontinuous system was used to promote adaptation to MISLS. Total organic carbon analysis and measurements of polysaccharides content were taken to establish carbon consumption by the bacterium. Moreover, MISLS was fully characterized by NMR and GPC prior and after the treatment to highlight any possible structural modifications.
In untreated LS, the bacterium primarily utilized the polysaccharide fraction for growth, but significant reduction of MISLS content was observed under both aerobic anaerobic conditions, confirming its capacity to metabolize the lignin fraction as the sole carbon source.
To deepen the understanding of lignin depolymerization by *R. palustris*, a bioinformatic investigation was also made and it resulted in possible metabolic gaps in guaiacyl unit's degradation pathway. Consequently, batch experiments were conducted to evaluate the bacterium's growth, with particular focus on the catabolic pathway including vanillin. Different concentrations of this substrate were tested, showing that, even though not efficiently, this compound is uptaken and possibly used as carbon source. However, increasing the substrate concentration can lead to a growth's inhibition effect. Overall, the results obtained suggest that lignin can sustain *R. palustris* growth when given as unique source of carbon.

Keywords: purple non-sulfur bacteria, Rhodopseudomonas palustris, lignin degradation, lignosulfonate, vanillin monomers

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PURPLE PHOTOTROPHIC PLATFORMS BRIDGING CO_2 AND WASTE STREAMS TO HIGH-VALUE CHEMICALS

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Increasing waste generation and sustained CO_2 emissions highlight a major limitation in current industrial processes, where significant fractions of carbon and reducing power in off-gases (CO, H_2) and sulfur-rich effluents (thiosulfate, $S_2O_3^{2-}$) remain underutilized or are emitted as secondary pollutants. While coupling CO_2 fixation with the direct use of these reductants represents a promising strategy, existing biotechnological platforms are constrained by low energetic efficiency, mass transfer limitations and reliance on organic co-substrates. Purple phototrophic bacteria (PPB) represent a compelling platform capable of overcoming these limitations. By combining infrared-driven metabolism with exceptional metabolic versatility, they enable efficient CO_2 fixation while addressing key energetic and mass transfer constraints that limit conventional chemolithotrophic and gas fermentation systems. At the same time, they can direct reducing power toward the synthesis of high-value isoprenoid- and quinone-derived compounds, such as coenzyme Q10 (CoQ10) and carotenoids, which are currently manufactured through resource-intensive heterotrophic or mixotrophic processes, thereby limiting overall sustainability. In this study, we demonstrate for the first time that PPB can sustain phototrophic growth on CO_2 while utilizing industrial waste streams as electron donors such as H_2, CO and $S_2O_3^{2-}$, coupled with the simultaneous production of CoQ10 and carotenoids. Through a combined genome-feature guided analysis and a stepwise experimental validation, we identified metabolically versatile strains capable of integrating CO_2 fixation with high-value metabolite synthesis. Among the most promising strains, *Rhodospirillum rubrum* DSM 467 and *Rhodospirillum rubrum* DSM 107 exhibited the highest CoQ10 production, with DSM 107 reaching up to 9.6 $mg_{CoQ10}\ g_{biomass}^{-1}$ growing under CO_2/H_2 conditions, exceeding reported values for natural sugar-feed producers and approaching those of engineered systems, while maintaining active carotenoid biosynthesis. In addition, *R. rubrum* DSM 467 exhibited efficient CO oxidation under syngas conditions via the water-gas shift reaction, revealing a tightly regulated, hierarchical substrate utilization strategy driven by energetic constraints. In contrast, *Marichromatium purpuratum* demonstrated the capacity for simultaneous oxidation of H_2 and $S_2O_3^{2-}$, enabling parallel input of electron fluxes into the photosynthetic electron transport chain and resulting in enhanced metabolic activity and growth. Overall, these results establish an integrated framework that directly links CO_2 capture with the production of high-value compounds from industrial waste streams, supporting the development of more sustainable and resource-efficient bioprocesses.

Keywords: CO_2 fixation, coenzyme Q10, syngas and sulfur bioconversion, PPB platforms, redox metabolism.

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SEWAGE TREATMENT AND BIOMASS RECOVERY USING FLUIDIZED CARRIERS WITH PURPLE PHOTOTROPHIC BACTERIA

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Conventional sewage treatment using the activated sludge process achieves high treatment performance but requires substantial aeration energy and incurs high costs for excess sludge handling. Purple phototrophic bacteria (PPB) based wastewater treatment has attracted attention as a low-energy alternative capable of simultaneously removing organic matter, nitrogen, and phosphorus, while enabling the recovery of valuable biomass with potential use as feed and bioproducts. However, PPB exhibit poor settleability, which necessitates the use of costly auxiliary systems such as membrane separation and centrifugation for biomass harvesting. As an alternative, microbial carriers can promote adhesion by retaining biomass and provide a more cost-effective separation solution such as scrapping and squeezing. In this study, PPB were evaluated for sewage treatment performance using fluidized carriers, included hydrophilic sponge (HS), commercial kitchen sponge (KS), loofah sponge (LS). Batch experiments were conducted in 120 mL serum bottles inoculated with preliminarily cultured PPB and fed with synthetic sewage as the substrate, under a light intensity of 15 W m^{-2} for 4 days. The carrier filling ratio was set at 10% (v/v) based on apparent volume. The dissolved organic carbon (DOC) removal efficiencies were relatively high for HS (83%) and KS (84%), while LS tended to be lower (72%). In contrast, the nutrient removal efficiencies showed to be significantly higher in the LS (dissolved total nitrogen: 58%, PO_4^{3-}: 55%). Carbon elution from the natural fibers of LS (e.g., cellulose and hemicellulose) was observed, resulting in slightly lower DOC removal efficiency. This additional carbon supply likely enhanced nitrogen and phosphorus removal via PPB assimilation. The relatively smaller pore size (9 µm) and hydrophilic nature of HS promoted biomass adhesion, resulting in higher biomass recovery (1.7 mg-SS cm^{-3}). The LS also retained a certain amount of biomass (0.9 mg-SS cm^{-3}). In conclusion, the effectiveness of HS was confirmed, and LS was shown to have potential to be a promising natural carrier.

Keywords: Moving-bed reactor, Microbial adhesion, Carbon release

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COUPLING ELECTROCHEMICAL CO_2 REDUCTION TO FORMATE WITH PURPLE PHOTOTROPHIC BACTERIA FOR SINGLE-CELL PROTEIN PRODUCTION

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Anthropogenic CO_2 emissions are a major driver of climate change, prompting the development of technologies that can convert CO_2 into valuable products within a circular carbon framework. Among these, electrochemical CO_2 reduction (eCO_2RR) has gained attention because it operates under mild conditions, can be coupled with biological processes, and enables the selective production of formate. Formate is a particularly appealing liquid intermediate, as it can be readily utilized in bioconversion pathways. In this context, purple phototrophic bacteria (PPB) have emerged as promising candidates for single-cell protein (SCP) production due to their high protein content, typically ranging from 40% to 70% of their biomass. The carbon derived from formate is oxidized to CO_2 and subsequently refixed through autotrophic pathways, making it a strategically valuable substrate for biomass generation and SCP production. Two configurations were evaluated for coupling eCO_2RR with PPB cultivation. In the two-step configuration, formate was first produced via eCO_2RR in a 250mL (graphite rod working electrode with indium catalyst, Ag/AgCl reference and platinum counter) under potentiostatic control at -2,2 V vs. Ag/AgCl in PPB medium supplemented with 0.05 M $NaHCO_3$ at 30°C. The resulting medium was used for PPB cultivation (100mL) under NIR light (850nm) using *Rhodopseudomonas palustris* DSM 126 as inoculum. In the integrated configuration, eCO_2RR and PPB growth were carried out simultaneously in a 350mL reactor (Figure 1). Synthetic formate was preliminarily assessed as a carbon source for growth and SCP production in batch and fed-batch mode, reaching up to 60% protein content. In the two-stage configuration, PPB medium composition affected eCO_2RR performance, with a maximum faradaic efficiency of 25±6% and formate production of 2.2±0.4 g/L in 22h (100 mg/L·h, current density (j) of 4.0 mA/cm^2) in the presence of essential nutrients and L-cysteine·HCl. The formate produced was subsequently used for SCP production in a separate batch reactor, yielding 0.7±0.1 gCOD/gCOD and 57±1% protein content. A sequential configuration was then tested in a single reactor, where formate produced was first produced over 6h (~385 mg/L, 64 mg/L·h, 4.3 mA/cm^2) (Figure 2), followed by inoculation, resulting in a formate removal rate of 23 mg/L·h and 64% protein content. Finally, full integration enabled simultaneous formate production (60.4 mg/L·h, j: 3.6 mA/cm^2) and consumption, achieving an average protein content of 51±4%. Full integration demonstrates for the first time stable and continuous PPB-based SCP production from CO_2 in a single reactor. The integrated eCO_2RR-PPB system showed biocompatibility and reproducible protein yields (0.4 gProt/gFormate). Formate (up to 2.2 g·L^{-1}) was successfully converted into biomass with up to 60% protein content, highlighting a circular and sustainable CO_2 valorisation route.

Keywords: Purple bacteria, CO_2 reduction, Single cell protein, electrochemical biological process
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Figure 1. Setup of the full integrated system

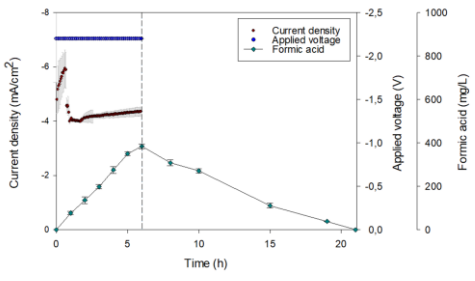


Figure 2. Current density and formate trend during PPB-eCO_2RR sequential configuration.

MOLYBDATE AS A SULPHATE REDUCING BACTERIA INHIBITOR DURING HYDROGEN PRODUCTION IN PNSB PHOTOBIOREACTORS

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Mixed cultures of enriched Purple Non-Sulfur Bacteria (PNSB) offer a versatile platform for wastewater valorisation through hydrogen production. However, its implementation can be hindered by the proliferation of Sulphate-Reducing Bacteria (SRB), which compete for organic substrates and can feed upon the hydrogen produced by the phototrophic consortia. Hence, the present study investigates the role of molybdate as a selective inhibitor of SRB in a batch system to maximize net hydrogen production using a mixed microbial culture enriched in PNSB.

The different batches were assayed in synthetic medium (1200 mg acetic acid/L and 230 mg SO_4^{2-}/L) at increasing molybdate concentrations (0, 0.5, and 1 mM Molybdate). Gas evolution was measured over time until a constant pressure was detected. Gas composition, substrate consumption, sulphate consumption, and biomass growth were analysed. The results showed active substrate consumption and hydrogen evolution (600-800 NmL H2/g COD consumed) across the different conditions, with slight differences in overall production and sulphate consumption. While, according to the literature, molybdate above 0.25 mM was expected to halt SRB activity, batch tests revealed a modest inhibitory effect in the PNSB-SRB consortia, with some H_2S production persisting even at 1 mM molybdate. Thus, SRB were active across all tested concentrations, underscoring the system's transient nature.

Nevertheless, this study provides general insight into the role of molybdate in SRB inhibition and shows that short-term batch assays may not be sufficient to assess the PNSB-SRB balance. This supports the need for future experiments with semi-continuous operating systems, where long-term selective pressure might be better for observing SRB suppression. This work set the basis for designing a more robust PPB-based system aimed at recovering hydrogen from agro-food waste streams that often contain significant sulphate content.

Keywords: Hydrogen, Enrichedconsortia, SRB, Sulphate, Molybdate.

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STUDY OF DIGESTATE VARIABILITY ON PURPLE PHOTOTROPHIC BACTERIA GROWTH: EFFECTS UNDER DIFFERENT ORGANIC LOADS AND LIGTHING CONDITIONS

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The use of the liquid fraction of digestate (LFD) remains a major challenge for the livestock and anaerobic digestion sectors, primarily due to its variable composition and limited biodegradability. Currently, purple phototrophic bacteria (PPB) are emerging as a promising biotechnological alternative for nutrient recovery and organic matter assimilation using digestate as cultivation media. However, their performance depends largely on substrate bioavailability and lighting conditions. This study evaluates the effect of digestate composition and lighting regimes on PPB growth using hydrolyzed LFD as a feedstock. Two digestates coming from the AD of cow manure, but showing contrasting organic loads were evaluated: low organic load (LOL; 5060 mg COD·L^{-1}, 1886 mg SCOD·L^{-1}) and high organic load (HOL; 18950 mg COD·L^{-1}, 9335 mg SCOD·L^{-1}). Alkaline hydrolysis increased SCOD by 13% and 17% for LOC and HOC, respectively, reflecting differences in the availability of particulate organic matter. To evaluate PPB photoheterotrophic growth, batch experiments were conducted under anaerobic conditions using 20% diluted LFD, dilution previously identified as optimal for PPB growth. The results showed a marked contrast in microbial performance. While HOL favored efficient growth (μ=1.3 d^{-1}; Y=0.73 g $COD_{biomass}$·g $COD_{removed}^{-1}$), LOL showed negligible growth (Y ≈ 0.01), despite its measurable SCOD. Unclear inhibition mechanisms in LOL prompted the evaluation of light intensity, supplementation of acetate and phosphate. Acetate emerged as the main factor enabling biomass development, indicating carbon availability as the primary limiting factor. This was confirmed in supplementation assays, where acetate restored PPB activity (μ= 0.93 d^{-1}, Y= 0.97), achieving removals of 78% SCOD, 41% NH_4^+-N and 99% PO_4^{3-}-P. Similar trends were observed when supplemented the digestate with raw manure as an alternative source of VFA. Light spectrum also played a key role. Infrared illumination (850 nm) resulted in significantly higher growth rates (μ = 0.93 d^{-1}) compared to UV-filtered white light (μ = 0.27 d^{-1}). However, biomass yield and nutrient removal efficiencies remained comparable, suggesting that light spectrum primarily affects kinetics rather than stoichiometry. These results highlight that digestate characterization for PPB applications must consider carbon speciation and bioavailability as key parameters for process optimization. This requires tailored strategies depending on substrate composition, where hydrolysis may be sufficient in some cases, whereas more recalcitrant LFD require external supplementation with readily biodegradable carbon sources. Raw manure is identified as a viable and low-cost alternative, although its effectiveness is strongly dependent on its VFA content.

Keywords: digestate, purple phototrophic bacteria, inhibition, carbon bioavailability, light spectrum

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FROM SUBSTRATE CONSUMPTION TO INTRACELLULAR STORAGE: SALINITY EFFECTS IN PPB MIXED CULTURES

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Salinity is usually seen as a limitation in biological wastewater treatment, yet its impact on microbial carbon processing remains poorly understood. This study investigated how salinity influences substrate utilization and carbon partitioning (mineralization vs. intracellular storage) in mixed cultures enriched in purple phototrophic bacteria (PPB). A saline-adapted PPB-enriched culture was exposed to six NaCl concentrations (0, 5, 10, 20, 35, 50 g L^{-1}) in parallel 2-L batch assays. Four pulses of acetic acid (200 mg C-HAc L^{-1} each) were supplied in the absence of nitrogen to limit biomass growth. Cultures were continuously illuminated with incandescent lamps (111.5 ± 3.5 W m^{-2}). The experiment was run for two weeks, during which substrate consumption, soluble inorganic carbon (IC), and intracellular polyhydroxyalkanoates (PHA) storage were monitored.

Increasing salinity reduced substrate uptake rates. Complete depletion of the supplied carbon was achieved at 0 and 10 g NaCl L^{-1}, whereas at higher salinities (35 – 50 g NaCl L^{-1}), uptake progressively slowed down, reaching around 10 mg C-HAc L^{-1} d^{-1} in the second week of the operation. Notably, higher salinities triggered a metabolic shift, as the ratio of soluble IC produced per C-HAc consumed (IC/HAc) decreased by 50 % at 50 g NaCl L^{-1} compared to low-salinity conditions. This reduction in mineralization was accompanied by enhanced PHA accumulation, reaching up to 75% (VSS). While PHA storage under non-saline conditions was transient, high salinity sustained elevated PHA levels comparable to those of pure cultures. Microbial analysis revealed that the community remained stable, with *Cereibacte*r dominating, suggesting that physiological adaptations rather than community shifts drove the observed changes. Overall, although salinity reduces process kinetics in PPB, it promotes intracellular carbon storage. This behavior highlights both constraints and opportunities for PPB-based resource recovery from saline wastewaters, providing new insights into PPB responses to osmotic stress.

Keywords: carbon partitioning, osmotic stress, PHA granules, resource recovery, substrate consumption.

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THE ROLE OF BIOCHAR IN MUNICIPAL WASTEWATER TREATMENT USING PURPLE PHOTOTROPHIC BACTERIA

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Conventional wastewater treatment has primarily focused on pollutant removal and water recovery, whereas recent approaches aim to reduce energy and chemical consumption while enabling resource recovery within a circular economy framework. Purple Phototrophic Bacteria (PPB) have emerged as a promising alternative mainly due to their metabolic versatility, which enables the simultaneous removal and recovery of carbon, nitrogen, and phosphorus, while producing high-value by-products and operating without the need for aeration, utilizing infrared (IR) irradiation. Despite these advantages, efficient nitrogen removal under low organic carbon conditions remains challenging, particularly due to limitations in coupling nitrification and autotrophic denitrification.

This study investigates PPB-based photo-bioelectrochemical systems for municipal wastewater treatment, focusing on the role of bio-based conductive materials, such as biochar, as electron mediators. The main objective is to evaluate whether conductive biochar can enhance nitrogen removal, process stability, and overall system performance under autotrophic conditions, with an emphasis on ammonia oxidation and denitrification mechanisms.

Laboratory-scale (700 mL), IR-illuminated photobioreactors packed with conductive biochar are operated under non-sterile, ambient conditions using PPB-enriched cultures derived from wastewater. A packed biochar column is immersed within the reactor to evaluate its role in facilitating electron transfer between redox zones. The system functions as a microbial electrochemical snorkel, enabling electron transfer across redox gradients and promoting the reduction of nitrite and nitrate. Key physicochemical parameters, including COD, nitrogen species, and redox potential, are monitored to assess process performance and stability. The results provide new insights into redox interactions in PPB-based systems and support the development of scalable, energy-efficient wastewater treatment technologies incorporating bio-based conductive materials.

Keywords: purple phototrophic bacteria, biochar, wastewater, microbial electrochemical snorkel

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HEALTH-SAFE SINGLE CELL PROTEIN PRODUCTION THROUGH *CUPRIAVIDUS NECATOR* AND *RHODOPSEUDOMONAS PALUSTRIS*

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Significant anthropogenic food waste and a global shift toward resource-intensive diets highlight the need for circular economy solutions like biorefinery platforms for single-cell protein (SCP) synthesis. This study evaluated the SCP potential of *Cupriavidus necator* type strain and *Rhodopseudomonas palustris* using acetic acid (HAc) at 0.5, 1.0, and 1.5 g/L. In *R. palustris*, the metabolic transition between NH_4 and N_2 fixation revealed that while biomass yields remained consistent, the HAc consumption rate for diazotrophic cultures was double that of NH_4^+-utilizing cultures, reaching about 990 mgCOD/h/L at 0.5 g/L. Peak biomass yields were observed at 0.5 g/L HAc, with diazotrophic *R. palustris* achieving the highest at 1.19 gCOD/gCOD. Conversely, Chemical Oxygen Demand (COD) mass balances for *C. necator* indicated substantial substrate deficits at 1.0 and 1.5 g/L, suggesting that the low yields recorded at these concentrations were likely influenced by the effect of substrate volatilization rather than changes on the metabolic flux. Variations in infrared irradiance (50–150 W/m²) for *R. palustris* showed no significant impact on performance compared to the 100 W/m² control. Finally, shotgun metagenomic sequencing of bacterial cultures grown in ultra-filtrated urban wastewater from a water resource recovery facility in Southern France, showed no significant increase in antibiotic resistance or virulence gene relative abundance compared to the initial conditions, indicating no ARG proliferation during growth. Quantitatively, *Rhodopseudomonas* showed the most promise for SCP production using either NH_4^+ or N_2. Qualitatively, no cultures evidenced ARG proliferation, and further analyses, such as amino acid profiling, are currently underway.

Keywords: Single Cell Protein, Biorefinery, PPB, Sustainability.

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PURPLE BACTERIA IMPROVE GROWTH, BRIGHTNESS, AND PHYSIOLOGICAL PERFORMANCE IN ORNAMENTAL KOI CARP

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Ornamental fish industry is a rapidly growing sector with high commercial value, where improvements in growth, pigmentation, and robustness are key nutritional targets. However, conventional pigment enhancers and fishmeal-based feeds face increasing sustainability and supply challenges, driving interest in alternative microbial ingredients. Purple bacteria (PB) are promising multifunctional feed sources, combining high quality protein with carotenoids and other bioactive compounds. Although PB-based diets have been associated with improved fish growth, their effects on pigmentation, health, and the mechanistic role of carotenoids remain largely unexplored.

This study evaluated the effects of the purple bacterium *Rhodobacter capsulatus* as a feed additive for koi carp (*Cyprinus rubrofuscus*). A 12 week feeding trial was conducted with 400 fish distributed among five dietary treatments: (i) a control diet without additives, (ii) a positive control supplemented with a pigment rich mixture of Chlorella spp. and Moringa (C-M), and (iii–v) diets containing 2%, 5%, or 10% purple bacteria (PB) biomass. Growth performance, feed conversion ratio, and coloration were assessed through biometric and image-based analyses. Metabolic performance and immune resilience were evaluated through respirometry, and *Flavobacterium columnare* challenge tests, while untargeted plasma metabolomics was used to investigate physiological responses.

PB supplementation improved fish performance across treatments. PB diets showed the highest specific growth rates and weight gain, with 2% inclusion performing best overall, while the C–M diet showed the weakest growth response. PB supplementation also altered pigmentation profiles, significantly increasing fish lightness and producing a brighter appearance, a desirable trait in ornamental koi. In contrast, the C–M diet increased color saturation but resulted in darker fish. Respirometry assays showed up to 59% higher UCrit values in PB fed fish compared with the C–M group (Figure 1 left). Plasma metabolomics further revealed xanthophyll-related metabolites (Figure 1 right), linking carotenoids with physiological response.

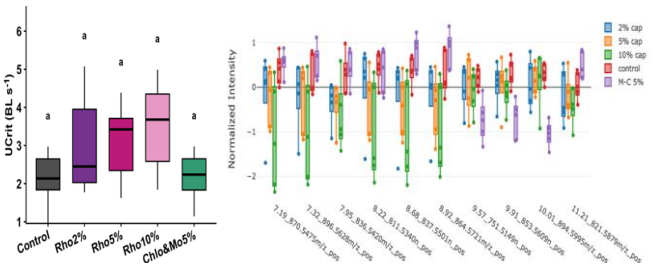


Figure 1. Critical swimming speed (UCrit) of koi carp (left) and differential plasma features detected by untargeted metabolomics,

Overall, PB emerged as a promising multifunctional feed ingredient for ornamental aquaculture, simultaneously enhancing growth, pigmentation, and physiological robustness.

Keywords: *Microbial protein, pigments, diazotroph*
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Acknowledgements: *BOF project PurpleHealth (CBE-JU project Purple4Life (FFI240198). L.D.A. is supported by FWO-Vlaanderen (grant no. 12A2925N).*

BENCHMARKING NITROGEN FIXATION IN PURPLE BACTERIA FOR MICROBIAL PROTEIN PRODUCTION

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Nitrogen fixation is a key process in ecosystem nutrient cycling and the main natural route converting atmospheric nitrogen (N_2) into biomass. In contrast, industrial nitrogen fertilizer production through the Haber–Bosch process is highly energy intensive and generates substantial CO_2 emissions, motivating the search for biological alternatives for sustainable protein production. Diazotrophic microorganisms are attractive because they produce nitrogen rich biomass directly from N_2, despite the high energetic demand of nitrogen fixation. Purple phototrophic bacteria are particularly suited for this process, as light driven cyclic photophosphorylation efficiently supplies ATP while enabling high conversion of organic carbon into biomass. However, whether N_2 fixation can sustain high PPB productivity and how it affects biomass composition remain unexplored.

Four PPB species, *Rhodopseudomonas palustris, Rhodobacter capsulatus, Rhodospirillum rubrum,* and *Cereibacter sphaeroides*, were cultivated with acetate under photodiazotrophic conditions, using N_2 as the only nitrogen source. Their growth was compared with standard photoheterotrophic cultivation (NH_4Cl as N source). *C. sphaeroides* showed the strongest response, with a 28% higher growth rate than under ammonium-fed conditions. This strain was then selected for continuous operation in a 7 L CSTR photobioreactor operated at 28 °C, pH 7, 50 W m^{-2}, and an acetate loading rate of 8 g COD L^{-1} d^{-1}. Photoheterotrophic and diazotrophic regimes were compared at HRTs of 2, 1.5, and 1 d.

Diazotrophic growth did not compromise biomass formation. In batch tests, protein content remained comparable to ammonium-fed cultures, while PHA accumulation decreased and pigment production increased. The amino acid profile also improved, with the essential-to-non-essential amino acid ratio reaching 54%. In continuous photobioreactor operation, *C. sphaeroides* reached up to 3.5 g VSS L^{-1} d^{-1}, with 65% protein and 0.8% carotenoids. At HRT 1.5 d, diazotrophic growth achieved similar biomass productivities to photoheterotrophic growth, around 1.9 g VSS L^{-1} d^{-1} (Figure 1), with only a moderate reduction in protein content. The maximum nitrogen fixation rate reached 108 mg N L^{-1} d^{-1}.

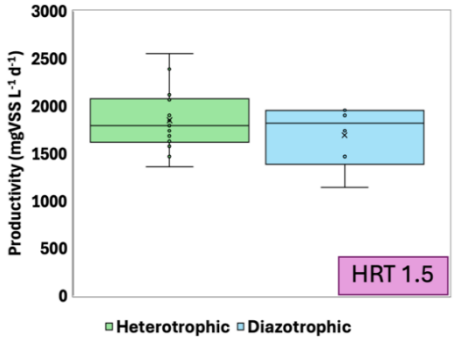


Figure 1: Biomass productivity of Cereibacter sphaeroides *under photoheterotrophic and photodiazotrophic growth.*

Diazotrophic PPB cultivation is not a productivity penalty. It can sustain high biomass formation while removing the need for external nitrogen addition. This makes PPB a promising platform for nitrogen independent microbial protein production.

Keywords: Microbial protein, pigments, diazotroph
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EFFECT OF ABIOTIC STRESS IN LATE EXPONENTIAL PHASE ON LIPIDS, COENZYME Q10 AND CAROTENOIDS PRODUCTION BY THE PURPLE BACTERIA *CEREIBACTER SPHAEROIDES*

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Coenzyme Q10 (Q10) and carotenoids (CRT) are extensively used in the fields of medicine, cosmetics, food, and health care due to their antioxidant properties. Low Q10 levels are associated with age-related and cardiovascular diseases, while carotenoids have been linked to protection against macular degeneration, cancer, obesity, neurodegeneration, and skin ageing. To meet the increasing demand for Q10 and CRT, sustainable and effective alternatives to conventional production methods are urgently needed. Purple bacteria (PB) offer a promising alternative: they grow phototrophically with near-unity carbon efficiency and naturally accumulate Q10 and CRT within cell membranes, where lipid composition, particularly vaccenic acid, plays a key structural and functional role.

Cereibacter sphaeroides was cultivated in 500 mL batch bottles under anaerobic conditions at 28 °C and 45 W m^{-2}, using acetate (1 g COD L^{-1}) as carbon source. Growth (OD), COD consumption, CRT and bacteriochlorophyll (BChl) (UV–Vis), Q10 (HPLC), and lipid content were monitored**.** At approximately 90% of the exponential growth phase, abiotic stress conditions were applied, including pH shifts (pH 4.5 and 9.5), temperature shocks (4 °C and 42 °C) and dark conditions (no light) for 2, 4 and 6 hours.

Results were strongly influenced by the applied abiotic stress (Figure 1). In the control, Q10, CRT and BChl content is 5.9 ± 1.1, 5.23 ± 0.3 and 16.7 ± 2.3 mg/g DCW respectively. After abiotic stress, notable observations include complete loss of bacteriochlorophyll at low pH and a ~40% increase in coenzyme Q10 at low temperature (8.3 ± 0.57 DCW). Lipid composition is expected to change under tested conditions, and data processing is still ongoing.

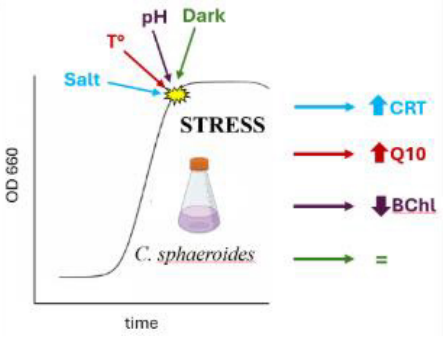


Figure 1. Effect of abiotic stress at the end of exponential growth on Q10, CRT and BChl.

This study examines the relationship between membrane lipids, carotenoids, and coenzyme Q10 in PB before and after abiotic stress. Applying stress during the final ~10% of cultivation can enhance productivity without affecting growth, offering a simple route to lower batch production costs.

Keywords: purple phototrophic bacteria, coenzyme Q10, carotenoids, abiotic stress.

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REMOVAL OF PSEUDOMONAS AERUGINOSA AND *E. COLI* BY PHOTOGRANULES IN A BUBBLE COLUMN PHOTOBIOREACTOR

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Sustainable wastewater treatment technologies have increasingly focused on nature-based and energy-efficient systems. Photogranule-based reactors have demonstrated strong potential for nutrient and organic matter removal; however, their capacity for pathogen removal under realistic operational conditions remains poorly understood. the removal of *Escherichia coli* and *Pseudomonas aeruginosa* from real municipal wastewater using a bubble column photobioreactor containing photogranules operated as a sequencing batch reactor. Two hydraulic retention times (HRTs), 1.0 and 1.7 days, were tested using effluent from an upflow anaerobic sludge blanket (UASB) reactor under controlled temperature (25±2°C) and artificial illumination (~205 µmol photons m^{-2} s^{-1}, 16:8 h light–dark cycle). Pathogens were quantified using selective chromogenic media. A total of 11 sampling campaigns were conducted after steady-state conditions were achieved. The reactors had a total volume of 2.5 liters (0.34m height and 0.096m internal diameter) and contained 1.7 liters of feed (influent) wastewater. They were operated as sequencing batch reactors. Influent concentrations averaged 4.55×10^8 CFU·100 mL^{-1} for *Pseudomonas aeruginosa* and 4.59×10^5 CFU·100 mL^{-1} for *Escherichia coli*. The shorter HRT (1.0 day) resulted in higher removal efficiencies, achieving 2.66 and 3.27 log reductions for *Pseudomonas aeruginosa* and *E. coli*, respectively, compared to 1.36 and 2.98 log reductions at 1.7-day HRT. These findings contrast with previous studies reporting optimal pathogen removal at significantly longer HRTs (~5 days), indicating that photogranule-based systems can achieve efficient pathogen removal under substantially reduced retention times. Pathogen concentrations associated with photogranule biomass remained low and showed no statistically significant differences between operational conditions ($p > 0.05$), suggesting limited accumulation within the granules and potential advantages in terms of biosafety. Overall, the results demonstrate that photogranules represent a promising and compact alternative for pathogen removal in decentralized wastewater treatment systems, particularly under short hydraulic retention time

Keywords: microalgae, wastewater treatment, pathogen removal.

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ASSESSING THE SYMBIOTIC ENHANCEMENT OF PPB SUPPLEMENTATION IN A MICROALGAL COLUMN PHOTOBIOREACTOR FOR HIGH STRENGHT WASTEWATER TREATMENT

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The treatment of high-strength wastewater using photobioreactors (PBRs) is often constrained by ammonium (NH_4^+) toxicity, which inhibits microalgal growth and compromises nutrient removal efficiency. Purple phototrophic bacteria (PPB) are known for their high tolerance to NH_4^+ and metabolic versatility, which could improve the overall treatment performance when co-cultured with microalgae. This study evaluated in a 22 L stand-alone column algal-bacterial PBR, the impact of PPB supplementation during the treatment of high strength synthetic wastewater (SWW), focusing on carbon and nitrogen removal. Following PPB supplementation, TOC removal efficiency unexpectedly decreased by 45% compared to the previous stage. In contrast, inorganic carbon (IC) removal increased from 32.8% to 74.2%. This shift may be explained by the use of biodegradable organic carbon as an electron sink, prompting PPB to enhance IC uptake to maintain metabolic equilibrium. On the other hand, PPB supplementation did not improve NH_4^+ or total nitrogen (TN) removal. Instead, NH_4^+ accumulated within the PBR after PPB addition, indicating a disruption of the nitrogen assimilation capacity of the established microalgae-bacteria consortium. These findings were not in agreement with the expected synergistic effects reported in sequential systems, where PPB reduce organic load and nitrogen toxicity prior to microalgae polishing. In this configuration, competitive or inhibitory interactions likely override the potential benefits. In conclusion, further studies on synergy mechanisms and operational conditions are required to establish effective PPB–microalgae integration strategies.

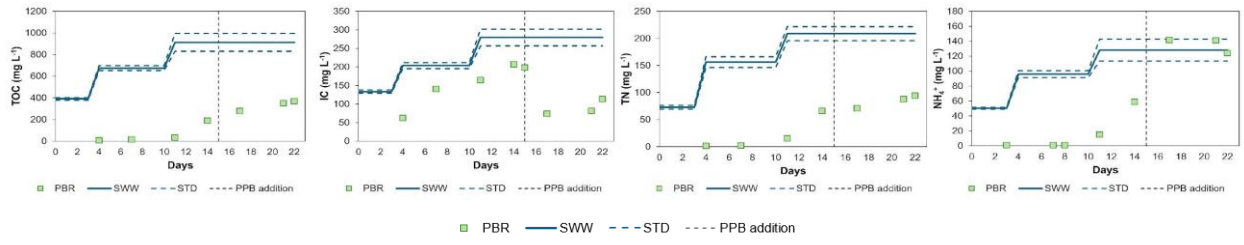


Fig. 1: Time course of a) TOC, b) IC, c) TN and d) NH_4^+ concentrations in the inlet and effluente of the PBR. Vertical dash line indicates PPB addition, while horizontal lines stand for standard deviation.

Keywords: Purple phototrophic bacteria, microalgae-bacteria consortium, high-strength wastewater

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COUPLING WET AIR OXIDATION AND PPB PHOTO-BIOELECTROCHEMICAL SYSTEMS FOR REFINERY SLUDGE VALORIZATION

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Oily sludge generated in petroleum refineries is a hazardous residual stream with high organic load, complex composition and limited sustainable treatment alternatives. This work evaluates a light-driven photo-biorefinery strategy coupling wet air oxidation (WAO) with purple phototrophic bacteria (PPB) operated in photo-bioelectrochemical reactors to partially convert the oil hydrocarbons into high value-added compounds.

WAO was applied as a pre-treatment to oxidize and solubilize high molecular weight hydrocarbons from refinery oily sludge and produce an aqueous phase suitable for biological conversion. The resulting effluent, with high soluble chemical oxygen demand (SCOD ≈11.4 g L^{-1}) and ammonium content (NH_4^+ ≈0.84 g L^{-1}), was diluted to 33% (v/v) to prevent microbial inhibition while maintaining substrate availability and used as substrate for an enriched natural PPB culture. The biological stage was carried out in H-type photo-microbial electrolysis cells equipped with graphite anodes polarized at +0.2, +0.4, +0.6 and +0.8 V vs Ag/AgCl with platinized titanium mesh as cathodes and under infrared illumination. Open-circuit reactors (OCP) were operated as controls.

Anodic polarization accelerated soluble organic conversion. After four days, SCOD removal increased from 22% in OCP to 35–43% in polarized reactors after 4 days, with +0.8 V showing the highest removal at this stage and reaching the maximum overall removal of the assay by the end of the experiment (52%). Final residual SCOD was 21-32% lower than under OCP, and polarized reactors maintained late-stage activity after OCP approached near-zero consumption. NH_4^+ removal increased from 12% in OCP to 27-34% in the polarized conditions.

Valorization followed a different trend. Planktonic biomass peaked at +0.4 V (800 ± 100 mg VSS L^{-1}), whereas +0.8 V decreased to 660 ± 50 mg VSS L^{-1}. Apparent biomass yield was 12% higher than OCP at +0.4 V but 32% lower at +0.8 V, confirming a shift from anabolic allocation to electrode respiration. Biofilm formation and the biomass-normalized carotenoid index were highest at +0.2 V. Thus, +0.8 V favoured organic conversion and ammonium removal, whereas +0.2-+0.4 V favoured biomass partitioning and pigment-oriented valorization.

Keywords: Purple phototrophic bacteria; Wet air oxidation; Photo-bioelectrochemistry; Refinery sludge; Carotenoids

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PPB ENABLED BIOHYDROGEN PRODUCTION FROM HYDROTHERMAL LIQUEFACTION WASTEWATER FOR LOW-CARBON BIOCRUDE UPGRADING IN A SOLAR-ASSISTED PHOTOREFINERY

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Purple photosynthetic bacteria (PPB) have unique value in sustainable biorefinery systems because they can convert organic wastewater and process side-streams into renewable biohydrogen, providing a key biological hydrogen source for low-carbon fuel production. Hydrothermal liquefaction (HTL) can convert wet organic wastes such as food waste into biocrude without energy-intensive drying; however, the process still faces two fossil-dependent bottlenecks. First, the high temperature and high pressure HTL reaction is commonly driven by conventional electricity or fossil energy. Second, the resulting biocrude typically has high oxygen content, low heating value, and poor stability, requiring hydrodeoxygenation upgrading, while the hydrogen used for upgrading is mainly derived from fossil resources. To address these challenges, this study placed PPB-driven biohydrogen production at the core of a solar-assisted photobiorefinery, converting HTL wastewater into renewable hydrogen for biocrude upgrading while using solar energy to replace conventional energy input.

Food waste was first converted into biocrude through HTL, and the HTL aqueous phase was further used as a substrate for dark fermentation coupled with PPB based photo-fermentation. Through light-oxygen regulation and Fe_3O_4 carrier-assisted granulation, the PPB system enriched purple hydrogen-producing bacteria to 70.2% and enabled stable operation of the up-flow photobioreactor for more than 200 days. Compared with a conventional single-stage completely mixed reactor, the integrated dark–photo fermentation system increased organic loading, hydrogen production rate, and substrate conversion by 107%, 60%, and 234-301%, respectively. Biohydrogen production reached 34.5 L/kg food waste, closely matching the hydrogen demand for catalytic biocrude upgrading of 34.1 L/kg food waste, enabling 100% replacement of external hydrogen.

The internally supplied biohydrogen supported deep hydrodeoxygenation of biocrude. After upgrading, the higher heating value increased from 35.10 to 48.95 MJ/kg, hydrogen content increased from 10.48 to 13.90 wt%, and oxygen content decreased from 23.49 to 0.48 wt%. The solar energy system achieved a solar contribution of 96.7% and waste-heat recovery efficiency of 56.6%. Life cycle assessment showed that the solar assisted PPB-integrated pathway reduced the carbon footprint from 5088.927 to 8.235 kg CO_2 eq, corresponding to a 99.84% reduction. These results demonstrate that PPB serve as a central biological link in sustainable photorefineries by coupling HTL wastewater valorization, renewable hydrogen generation, and near-zero-carbon biocrude upgrading.

Keywords: Purple photosynthetic bacteria; Hydrothermal liquefaction wastewater; Biohydrogen-driven biorefinery; Low-carbon biocrude upgrading; Life cycle carbon reduction

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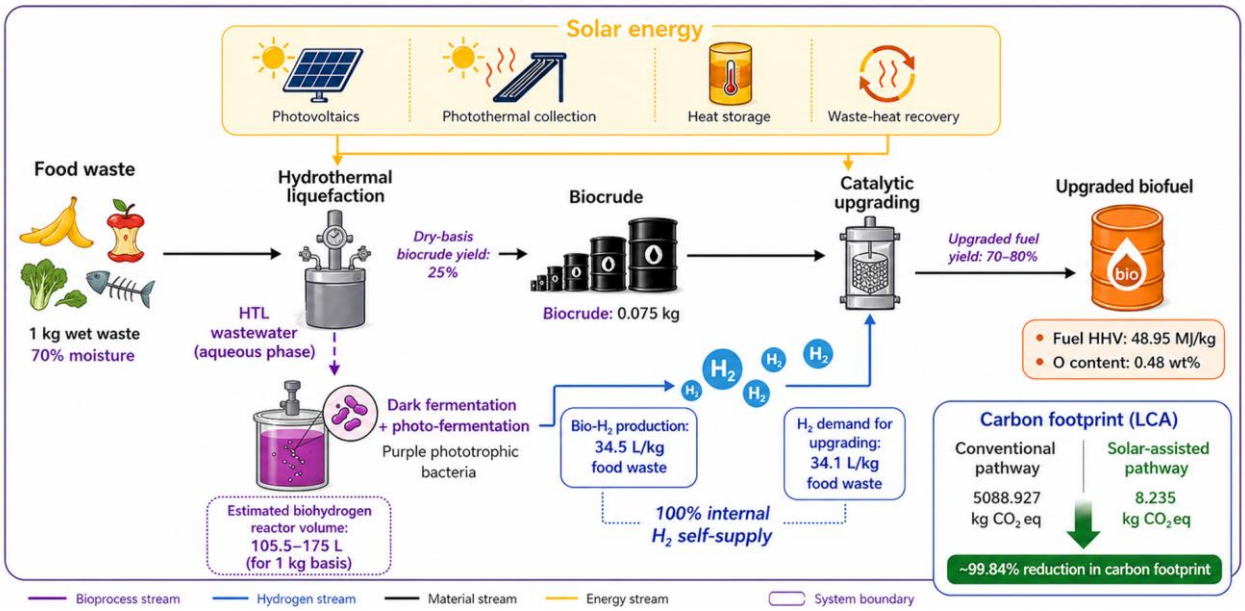


Figure 1. *PPB-enabled solar-assisted photobiorefinery for low-carbon biocrude upgrading.*

SHIFTING PARADIGMS FROM ENERGY DOWNCYCLING TO NUTRITIONAL UPCYCLING: OPTIMISATION OF BEET MOLASSES FOR *RHODOSPIRILLUM RUBRUM* BIOMASS AND HIGH-VALUE METABOLITES

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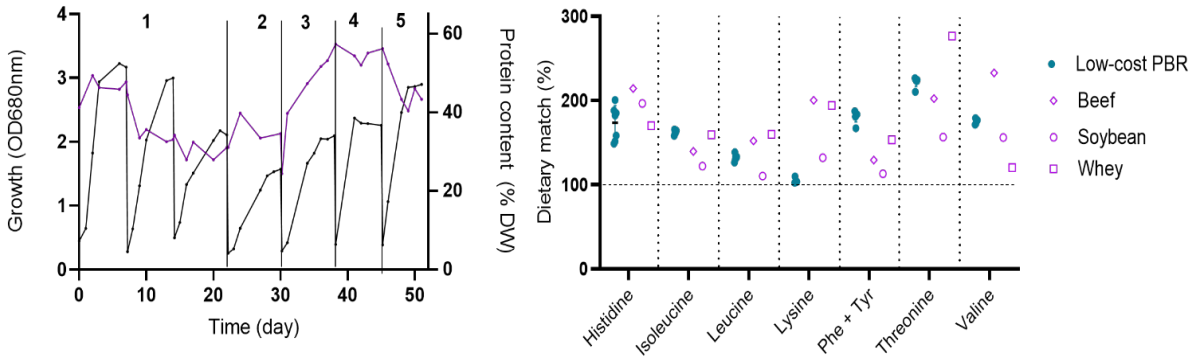
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The global transition toward a circular bioeconomy necessitates a fundamental rethink of industrial co-products. Currently, sugar beet molasses is predominantly directed toward low-value applications, such as animal feed or bioenergy production. This conventional approach represents a clear case of downcycling, where a high-potential carbohydrate stream is utilized for its simplest energetic or caloric value. In contrast, this study proposes a high-impact upcycling strategy by transforming molasses into premium microbial protein and bioactive compounds (*i.e.* carotenoids and co-Q10), using a specialised, glucose-acclimated strain of the purple non-sulphur bacterium *Rhodospirillum rubrum*. To unlock the full biological potential of this feedstock, we employed a D-optimal response surface methodology to design a precision-engineered growth medium. Our optimization process focused on seven critical nutritional pillars: major macronutrients, including ammonium, phosphate, sulphate and bicarbonate), alongside essential micronutrients such as magnesium, manganese and calcium, supplemented with enzymatic invertase. By systematically fine-tuning these components, we successfully identified the specific conditions required to maximize carbon assimilation while ensuring a stable, robust, and reproducible production process at scale. Under these optimized conditions, the process yields a high-density biomass with a protein content exceeding 55% and an exceptional essential amino acid profile (EAAI>1). Furthermore, this nutritional upcycling framework allowed for the identification of optimal parameters that significantly favour the synthesis of high-value secondary metabolites, specifically Coenzyme Q10 and carotenoids. By prioritizing the production of high-quality biomass over traditional photofermentative hydrogen recovery, this research provides a scalable and economically attractive roadmap for the sugar industry. It demonstrates how industrial residues can be strategically reintegrated into the human food and nutraceutical chains, effectively aligning resource efficiency with global nutritional security.

Keywords : Nutritional Upcycling; Rhodospirillum rubrum; Medium optimisation; Coenzyme Q10; Carotenoids

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Protein content (A) and dietary match (B) of Rhodospirillum rubrum MG following medium composition optimisation. Bacterial growth is represented by a solid black line, while protein content is shown as a solid purple line

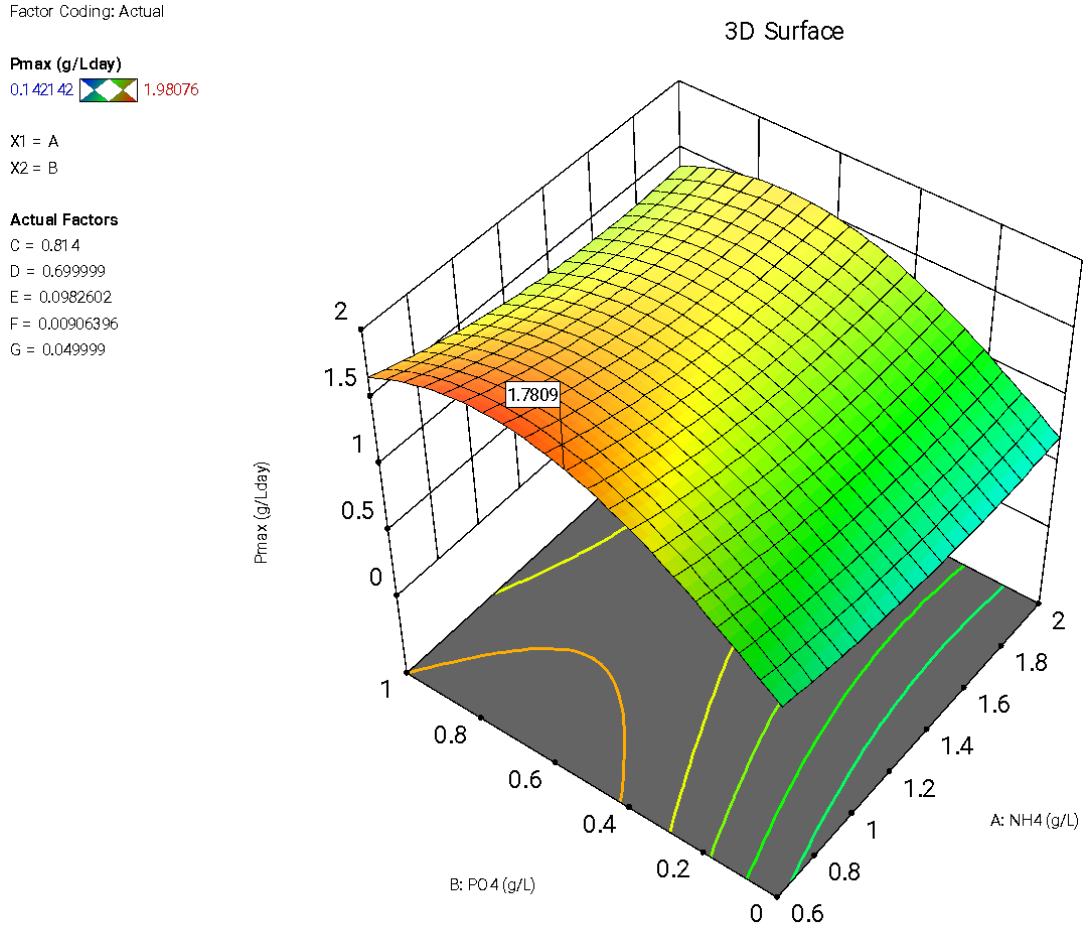


Figure 2: *3D surface plot of the scalar field showing spatial variation across the domain. The surface is colour-mapped according to magnitude, ranging from low values (blue) to high values (red), with a peak value around 1.78 indicated. The projection onto the base plane includes contour lines that highlight gradients and level sets of the field.*

Section 2.
BIOREFINERIES BASED ON MICROALGAE OR CYANOBACTERIA

BIOFILM PHOTO-CULTIVATION FROM ANAEROBIC SLUDGE USING ORGANIC OR INORGANIC CARRIERS

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Anaerobic digestate represents a nutrient-rich medium for microalgal cultivation, offering significant potential for simultaneous biomass production and nutrient recovery. However, its application is often limited by its darker colour, elevated ammonia concentrations, and the presence of inhibitory compounds. This study evaluated the production of a microalgal biofilm system using novel carrier materials to enhance biomass productivity and waste treatment performance. Microalgae growth in anaerobic digestate was first studied at different dilutions. The best production of 0.6g/L was obtained with a dilution that enabled a 125 mg/L ammonia concentration. Experiments were conducted over 9 days under mixotrophic and heterotrophic conditions using organic carriers (rice hulls) and inorganic carriers (K1 media) at a 12.5% fill ratio. Microalgal growth was monitored using optical density and attachment efficiency measurements, while treatment efficiency was assessed through ammonia, nitrate, phosphate, and chemical oxygen demand removal. The highest biomass productivity was 0.12 g/L/day and was achieved under mixotrophic conditions with the organic carrier, indicating that the combined utilisation of light and organic carbon enhanced growth. Furthermore, the system achieved substantial pollutant removal, reaching maximum removal efficiencies of 66.3 ± 12.2% (ammonia), 68.3 ± 13.9% (nitrate), 89.3 ± 9.2% (phosphorus), and 74.9 ± 12.1% (COD). The organic carrier further promoted biofilm formation and attachment efficiency compared to the inorganic carrier. Results indicated that the organic carrier outperformed the inorganic carrier across all key performance indicators, including biomass yield, attachment efficiency, and nutrient removal, highlighting its suitability for microalgal biofilm systems. Overall, the findings demonstrate that microalgal biofilm systems using organic carriers offer a promising and sustainable approach, whilst being a cost-effective solution for anaerobic digestate treatment and resource recovery.

FROM MICROALGAE SIDE-STREAMS TO POLYHYDROXYALKANOATES: CLOSING THE BIOREFINERY LOOP

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Microalgae-based biorefineries generate substantial side-streams whose underutilization limits overall process efficiency and sustainability. Within a circular biorefinery framework, these residual streams can be valorized into high-value products such as polyhydroxyalkanoates (PHAs), biodegradable polymers with properties comparable to conventional plastics.
In this study, microalgal side-streams were integrated into a three-stage process comprising acidogenic fermentation for volatile fatty acid (VFA) production, microbial enrichment of a mixed microbial culture (MMC), and PHA accumulation. Four up-flow anaerobic sludge blanket (UASB) reactors were operated under acidic (pH 4.8–5.5) or alkaline (pH 10–11) conditions and fed with acid (pH 2)- or alkaline (pH 11)-pretreated microalgae streams to generate VFA-rich effluents with distinct profiles. All acidogenic reactors were maintained at 35 °C with a hydraulic retention time of 1 day, while the organic loading rate increased gradually from 5 to 15 $gCOD.L^{-1}.d^{-1}$. The resulting VFA profiles showed distinct distributions of hydroxybutyrate (HB) and hydroxyvalerate (HV) bioprecursors. Acidic fermentation produced VFA streams containing approximately 60% HB and 40% HV precursors, whereas alkaline fermentation generated VFA streams containing 80% HB and 20% HV precursors, regardless of the microalgae pre-treatment. These differences were reflected in the composition of the PHA subsequently produced using a culture previously enriched in PHA-producing microorganisms: VFA streams obtained under acidic fermentation yielded polymers richer in HV (43% HB/57 %HV), while VFA streams from alkaline fermentation produced polymers with higher HB content (75% HB/25% HV). These results demonstrate that acidogenic fermentation pH plays a more decisive role than microalgae pre-treatment conditions in steering precursor distribution and, consequently, in shaping the final polymer composition and properties.
This work highlights the potential of integrating side-streams valorization into microalgae biorefineries, enabling flexible and tailored biopolymer production while improving overall resource efficiency and circularity.

Keywords: biorefinery; microalgae side-streams; polyhydroxyalkanoates

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EFFECT OF SUPPLEMENTING DIFFERENT VFA ON POLYHYDROXYALKANOATES CO-POLYMERS PRODUCTION USING CYANOBACTERIA

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Over the past decade, biobased and biodegradable plastics such as polyhydroxyalkanoates (PHAs) have gained attention as sustainable alternatives to petroleum-based plastics. Among them, the co-polymer, PHBV stands out due to its improved flexibility, and broader processing range compared to PHB. Cyanobacteria can sustainably produce PHA using CO_2 and sunlight. However, PHBV synthesis requires supplementation with precursors (e.g., valerate or propionate), which can be derived from waste fermentation. Mixed cyanobacterial cultures may further reduce costs by enabling cultivation in open systems, avoiding sterilization steps. Nevertheless, the relationship between supplemented volatile fatty acids (VFAs) and copolymer composition remains unclear.

In this study, Design of Experiments (DoE) was used to relate VFA supplementation to copolymer production and to determine optimal VFA concentrations. Different mixtures of four VFAs were added to nutrient-depleted cyanobacterial cultures under darkness (hypoxia) at 30 °C. After 7 days, total PHA accumulated and the HV/HB ratio were quantified and optimized.

Butyrate and acetate were the main drivers of PHA accumulation, with valerate showing a moderate effect and propionate being non-significant. A significant four-way interaction indicated synergistic effects among all components. For HV content, valerate was the primary contributor, although acetate– or butyrate–propionate mixtures also enhanced copolymer formation. The best mixture to enhance PHB content and the HV/HB ratio in the polymer was a mixture of 30 and 70% acetate and valerate, respectively. Mixtures of 50% of acetate and propionate can also achieve a high HV/HB content (up to a 40%) with a higher content of PHA.

Producing PHBV without valerate supplementation could significantly reduce production costs, since valerate is more difficult to produce via fermentation and is more expensive in the market.

Keywords: PHA, co-polymers, Cyanobacteria, Volatile Fatty Acids

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IMMOBILIZED MICROALGAE-BACTERIA CONSORTIA FOR NUTRIENT REMOVAL FROM REAL WASTEWATER: COMBINED EFFECTS OF N/P RATIO AND PHOTOPERIOD

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The growing global pressure to develop sustainable wastewater treatment technologies has accelerated research into biologically driven systems which can reduce energy consumption and operating under variable environmental conditions. Among these, microalgae-bacteria consortia (MBC) have emerged as a highly promising alternative. Unlike single microalgal species, MBC integrates both photosynthetic microalgae and heterotrophic bacteria. This may help to allow the consortium to overcome physiological limitations. One of the most important parameters affecting nutrient removal in microalgae-based systems is the nitrogen-to-phosphorus (N/P) ratio. Another critical parameter affecting metabolic activity and thus system performance is the lighting regime. Thus, it is aimed to investigate the combined effect of N/P ratio and illumination period on immobilization of MBC and its nutrient removal. This study presents an integrated investigation of how the N/P ratio and photoperiod jointly shape biofilm formation, consortia stability, and nutrient removal performance. This study was conducted in semi-continuous photobioreactors (HRT of 8 days) inoculated with *Chlorella vulgaris* and activated sludge bacteria, using polypropylene biocarriers for immobilization and real municipal wastewater from the Ankara Central Wastewater Treatment Plant. Three N/P ratios (5, 7, and 9) and two photoperiods (12:12 h light/dark and continuous 24-hour illumination) were tested with analyses of total ammonia nitrogen (TAN), nitrite, nitrate, orthophosphate, optical density, and pH monitored throughout the study. Results indicated that both photoperiod and nutrient stoichiometry significantly influenced system dynamics. Regarding the N/P ratio, the highest suspended biomass production was observed at N/P 9 for continuous illumination. However, at N/P 9 for 12:12h illumination, negligible amount of growth and immobilization was observed. Interestingly, biofilm immobilization was highly effective at the lower N/P 5 ratio. No single configuration achieved simultaneous complete nitrogen removal and maximum phosphorus elimination. Phosphorus removal exceeded 90% under continuous illumination for all N/P ratios, which is likely to be driven by sustained luxury uptake. The 12:12 h regime, despite enabling better nitrogen cycling, led to higher residual phosphate. TAN was efficiently removed across all conditions through nitrification, with the highest kinetic rates under N/P ratio of 5. This study demonstrated that continuous illumination maximized biomass growth and phosphorus removal but inhibited complete nitrogen cycling by suppressing denitrification. Conversely, cyclic lighting provided a more balanced microbial environment that enabled effective denitrification. Furthermore, lower N/P ratios (N/P 5) promoted early biofilm stability through stress-induced attachment, while higher ratios (N/P 9) supported dense suspended growth and luxury phosphorus uptake.

Keywords: MBC, immobilization, photoperiod, Chlorella vulgaris, wastewater treatment

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ECTOINE PRODUCTION FROM METHANOTROPH–MICROALGAE SYSTEMS: CO-CULTURE VERSUS MONOCULTURE STRATEGIES UNDER SALINE AND BIOGAS-FED CONDITIONS

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Methane-rich biogas represents an attractive feedstock for the sustainable production of high-value biochemicals. Methanotrophs have recently emerged as a promising platform for converting biogas into bio-based chemicals such as the osmolyte ectoine, providing a cost-effective alternative to sugar-based processes. Lately, the co-cultivation of methanotrophs and microalgae ("methalgae" systems) has also gained attention by coupling CH_4 oxidation for ectoine synthesis with CO_2 fixation, O_2 generation, and enhanced gas transfer collectively improving system robustness and conversion efficiency under mesophilic conditions.

In this study, a marine methanotroph–microalgae co-culture and their corresponding monocultures were evaluated for ectoine production using CH_4 and CO_2 from synthetic biogas. Experiments were conducted in a 5 L bubble column photobioreactor (3 L working volume) operated in batch mode under saline conditions (3.0% NaCl). An osmotic shock (4.5% NaCl) was induced upon nitrogen depletion to stimulate ectoine biosynthesis. The methanotrophic consortium was dominated by *Methylobacter marinus*, while the algal consortium consisted mainly of *Nannochloris* sp.

The methanotrophic monoculture achieved an average CH_4 consumption rate of 3.1 $mg_{CH_4}\ L^{-1}\ h^{-1}$, with no detectable CO_2 uptake or O_2 generation. In contrast, the microalgal monoculture removed CO_2 and released O_2 at higher rates than those observed in the co-culture, approximately 20% and 9% higher, respectively. However, the methanotroph–microalgae co-culture exhibited an enhanced CH_4 oxidation rate of 3.9 $mg_{CH_4}\ L^{-1}\ h^{-1}$. Within this system, microalgae removed approximately 14% of the CO_2 released by methanotrophs while producing about 0.1 mg $O_2\ L^{-1}\ h^{-1}$. This coupling improved internal CO_2/O_2 exchange and helped maintain a stable pH around 6.8 throughout the process. Ectoine productivity was highest in the methanotrophic monoculture, reaching 127 $mg_{ectoine}\ g_{VSS}^{-1}$, but remained comparable in the co-culture with a maximum of 85.9 $mg_{ectoine}\ g_{VSS}^{-1}$. As expected from previous reports, no ectoine was detected in the microalgal batch due to their inherent inability to synthesize this osmolyte. Overall, these findings indicate that while methanotrophic monocultures achieve higher short-term yields, co-cultures offer a more balanced strategy by enhancing process stability and improving overall gas utilization efficiency.

Overall, the choice between monocultures and co-cultures depends on the primary objective: maximizing short-term productivity versus building a more robust, stable process. Thus, the culturing strategy is a key driver of ectoine biosynthesis and gas consumption in this biogas-fed methanotroph–microalgae system. While methanotrophic monocultures achieve higher yields, co-cultures provide a powerful approach to steer both main biogas-derived carbon flows (CH_4

and CO_2) toward compatible solute production in a single integrated system, supporting a more circular biorefinery concept for ectoine synthesis.

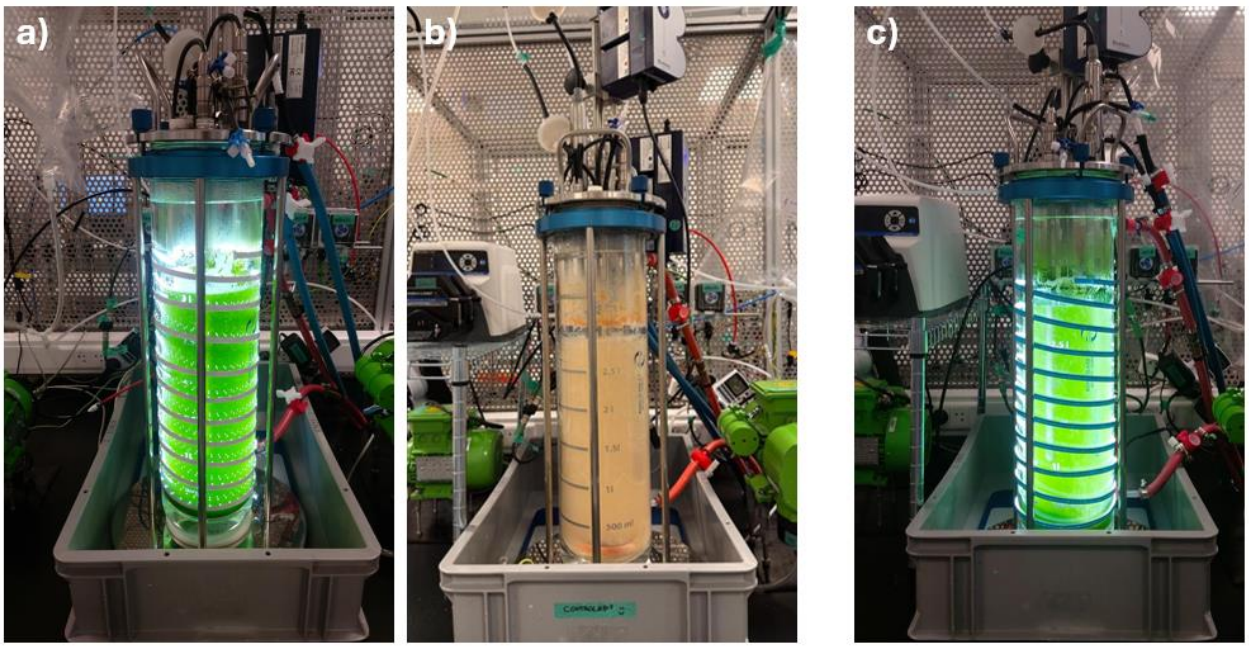


Figure 1. 5-L bubble column showing different batch runs: (a) microalgae, (b) methanotrophs, and (c) methalgae co-culture.

Keywords: methanotrophs, microalgae, co-culture, biogas, resource recovery

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MICROALGAL EXTRACTS AS PLANT BIOSTIMULANTS: EVIDENCE OF HORMONE-LIKE ACTIVITY

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Since the Industrial Revolution, human-driven greenhouse gas emissions have accelerated climate change, threatening agriculture and food security. Simultaneously, excessive and improper use of chemical fertilizers contributed to soil deterioration and environmental pollution. In this context, plant biostimulants offer a sustainable alternative, improving crop performance while reducing agricultural environmental impacts, and can be classified as microbial or non-microbial. Among these, microalgae are a promising and sustainable novel source, enabling the production of biostimulants while contributing to CO_2 mitigation, nutrient recovery and wastewater treatment. In particular, brewery wastewater represents one of the most relevant industrial effluents in terms of both economic relevance and production volumes. In this study, the biostimulant potential of microalgal extracts obtained from Scenedesmus spp. biomass cultivated in brewery wastewater was evaluated. Two biostimulants (US1 and US2) were obtained via probe sonication under identical conditions, differing only in mode: continuous (US1) or pulsed (US2). Yield was determined by measuring the total solids content of the final extract and comparing it to the initial amount of lyophilized biomass provided. The biostimulant potential was assessed through hormone-like activity bioassays, in which the extracts were compared to the activity of an untreated control (water) and the one of reference phytohormones, i.e. 6-benzylaminopurine (BAP), indole-3-butyric acid (IBA) and gibberellic acid (GA_3). US1 showed a slightly higher yield (31.3%) and increased adventitious root formation by ~44% at 1 g/L compared to the untreated control, demonstrating an auxin-like activity. US2 had a comparable yield (30.95%) and increased adventitious root formation by ~52% at 5 g/L, as well as stem elongation by ~22% at 2 g/L, demonstrating both auxin- and gibberellin-like activity. These findings highlight the potential of microalgae-based wastewater treatment processes as integrated biorefineries for the production of high-value biostimulants for sustainable agricultural applications.

Keywords: Biostimulants, Microalgal extracts, Scenedesmus spp, Hormone-like activity bioassays, Brewery wastewater.

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CO_2 VALORISATION VIA IMMOBILISED PHOTOSYNTHETIC MICROORGANISMS

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Climate change has made CO_2 reduction a central priority in global and EU policy, driving interest in biorefineries based on photosynthetic microorganisms that convert CO_2 into valuable compounds using light. Among these, carotenoid pigments are particularly attractive due to their antioxidant properties. However, conventional cultivation systems are limited by inefficient gas-liquid mass transfer and high culture medium requirements.

In parallel, the immobilisation of microorganisms in natural and synthetic matrices has attracted growing attention in recent years across a range of applications. A key advantage of maintaining microorganisms in a non-growing state is the ability to redirect their metabolic energy toward the production of valuable compounds, including biotransformation products and gases. However, one important aspect of the biorefinery potential of photosynthetic microorganisms has been largely overlooked in recent immobilisation strategies: pigment production. Many of these pigments are embedded within cellular membranes, necessitating cell disruption for efficient extraction. This limitation has hampered the adoption of immobilised systems for pigment production.

Within the CO_2MMITMENT project, we explore immobilisation strategies and non-destructive extraction methods for pigment recovery. Microorganisms are incorporated into synthetic latex to form biocoatings via desiccation or wet sintering. Extraction techniques such as milking, in situ solvent extraction, pulsed electric field, and supercritical fluid extraction are adapted for these systems, with pigments analysed by HPLC and NMR.

Complete desiccation within biocoatings proved successful only for the desert cyanobacterium *Chroococcidiopsis cubana* SAG 39.79. These biocoatings maintained CO_2 capture and O_2 production for more than 60 days. During this period, cell leakage was minimal, and the specific CO_2 uptake rate reached up to 1.9 g CO_2 g^{-1} dry biomass day^{-1}.

Keywords: Immobilisation, biocoating, microalgae, cyanobacteria, pigments

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PHOTOPERIOD-DEPENDENT GROWTH OF *CHROMOCHLORIS ZOFINGIENSIS* USING DHS-TREATED EFFLUENT

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Microalgae are widely recognized for their potential in biomass and value-added compound production using various nutrient media. Wastewater has been explored as a low-cost cultivation medium due to its high nutrient content. However, nutrient imbalance and the presence of suspended solids can limit microalgal growth. Treatment systems such as the downflow hanging sponge (DHS) reactor can enhance wastewater quality, making it more suitable for microalgal cultivation. *Chromochloris zofingiensis*, a freshwater microalga, has attracted considerable attention due to its ability to grow under diverse trophic modes and accumulate value-added products including protein, lipid, and carotenoids. However, the role of down flow hanging sponge system (DHS-) treated effluent for microalgal cultivation remains unclear. Thus, the purpose of this study was to evaluate the growth and carbon utilization of *C. zofingiensis* cultivated in DHS-treated effluent under different photoperiod conditions such as 12h L:12h D, 24h L, 18h L:6h D, 14h L:10h D and 24h D light and dark cycle respectively. The cells cultured in Modified Bristol's medium under identical conditions were used as control in this study. As a result, cells cultivated with DHS-effluent exhibited the highest specific growth rate (1.58 d^{-1}) under 18h L:6h D and the highest ash-free dry weight (0.25 g L^{-1}) under 14h L:10h D compared to other photoperiods. Higher dissolved organic carbon (DOC) removal was observed under dark (24D) and mixed photoperiod conditions (18L:6D and 14L:10D), whereas lower DOC removal occurred under continuous light (24L) and balanced light-dark cycles (12L:12D), suggesting reduced reliance on organic carbon under increased light availability. These observations suggest that cells exhibited mixotrophic metabolism, with greater dependency on organic carbon under low-light conditions and increased photosynthetic activity under higher light availability. Overall, these findings indicate that the growth of *C. zofingiensis* in DHS-effluent is governed primarily by light-dependent metabolic regulation rather than carbon limitation, highlighting the potential of DHS-effluent as a sustainable medium for microalgal cultivation.

Keywords: Anaerobic digestate, biomass, Chromochloris zofingiensis, Total dissolved carbon, Water Hyacinth juice

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SOIL EXTRACTS FOR GROWTH PROMOTION OF SLOW-GROWING VALUABLE MICROALGAE: TOWARDS MEDIUM DEVELOPMENT

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It is known that microalgae can produce high-value compounds; however, many of the 200,000 identified species remain commercially unexploited due to low growth and biomass yield. *Oocystella heteromucosa* and *Oocystis marina* are known to produce eicosapentaenoic acid (EPA) and lutein but exhibit slow growth. Soil-derived dissolved organic matter (DOM) has been considered a microalgal growth-promoting factor. DOM and other components, such as nutrients and metals, can be rich in soil extracts prepared at high temperatures. The influence of soil extracts prepared at different temperatures on slow-growing microalgal species remains unclear. Thus, this study evaluated the effects of soil extracts on the growth of *O. heteromucosa* and *O. marina* and identified the growth-promoting factors. Soil extracts were prepared by extracting red (R) or yellow (Y) soil from Okinawa, Japan at 25ºC, 105ºC or 121ºC for 1 h and re-heating at 121ºC for 0, 1, or 5 h. Later, soil extracts were replaced by standard humic substances (HS) and chemically derived metals to examine the effect of DOM. Compared to controls without soil extracts, the highest increase in specific growth rate was 2.6-fold for *O. heteromucosa* (Y, 121ºC, 5 h) and 2.1-fold for *O. marina* (Y, 25ºC, 5 h). These results suggest that growth-promoting components could be extracted at high temperatures for long heating time. Multiple linear regression analysis identified Mn, Fe, and P as potential growth-promoting factors, rather than DOM. When soil extracts were replaced by standard HS and chemically derived metals, it was found that the addition of HS and metals had a higher growth-promoting effect (1.2–1.8-fold), as compared to the one with addition of only metals (0.8–1.4-fold). These results indicate that soil-derived DOM, mainly HS, possibly plays an essential role in enhancing metal bioavailability and promoting slow-growing microalgal growth. In the future, soil extracts can be replaced by HS or other soil extracts rich in HS with metals for higher metal availability to the microalgae.

Keywords: Dissolved organic matter, Humic substances, Metals, Slow-growing microalgae, Soil extracts

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DEVELOPING PHOTOSYNTHETIC BIOCATALYST FOR SOLAR CHEMICAL PRODUCTION

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Photosynthesis-based bioproduction provides a promising strategy for converting CO_2 into valuable chemicals and fuels using light-driven processes. In this study, we design and evaluate solid-state photosynthetic cell factories based on genetically modified cyanobacteria immobilized in transparent hydrogel matrices approximately 200 μm thick.

For butanol production, cyanobacterial strains were engineered with a heterologous pathway regulated by an IPTG-inducible promoter, together with an aTc-inducible CRISPRi system targeting GltA to suppress growth. These cells were immobilized in Ca^{2+}-crosslinked alginate films (1.7% v/v) at different initial densities normalized to chlorophyll a content. Increasing the starting biomass did not improve volumetric butanol production, while biomass-specific productivity decreased at higher densities, indicating light limitation and self-shading in dense cultures. During cultivation, chlorophyll levels increased in low-density films and decreased in high-density films, converging after seven days. Confocal microscopy showed that cell distribution, initially uniform, became heterogeneous after approximately 14 days, suggesting localized light or CO_2 limitations.

For ethylene production, engineered strains expressing the ethylene-forming enzyme were incorporated into 3D-printed transparent scaffolds composed of photocurable wood-derived polymers, specifically TEMPO-oxidized cellulose nanofibrils (TCNF) and methacrylated galactoglucomannan (GGMMA). These materials supported stable ethylene production over seven days, with higher-charge formulations consistently outperforming lower-charge variants. This result highlights the importance of tuning scaffold properties to improve productivity.

Future work will focus on scaling these systems to continuous operation by improving light distribution and mass transfer through advanced cellulose-based materials, along with developing photobioreactors tailored to immobilized photosynthetic platforms.

Keywords: Butanol, Ethylene, Immobilization, Alginate, TCNF

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SEMICONTINUOUS CULTIVATION OF AN ETHIOPIAN STRAIN *LIMNOSPIRA FUSIFORMIS* USING ANEROBIC DIGESTION EFFLUENT

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Limnospira fusiformis has strong potential for mass production due to its rapid growth. However, large-scale cultivation of *L. fusiformis* largely depends on nutrient supply, particularly nitrogen; however, its high-cost limits economic feasibility. Anaerobic digestion effluent (ADE) from water hyacinth has emerged as a low-cost and sustainable alternative due to its high ammonium nitrogen. Nevertheless, its low pH and elevated ammonium levels make it unsuitable for direct application, as *L. fusiformis* thrives under alkaline conditions. Therefore, pH treatment is necessary to improve ADE suitability as a nutrient source. In this study, ADE was first treated at two different pH levels (9 and 10), and later dilutions (0-, 2-. 4- and 6- fold) were considered to determine optimal conditions for the cultivation of *L. fusiformis* was cultured using a column reactor in a semicontinuous cultivation under controlled conditions (30 °C and 400 µmol m^{-2} s^{-1}). The standard SOT medium was used as a control. It was found that *L. fusiformis* reached the maximum biomass yield of approximately 1.2 g L^{-1} in all ADE treatment conditions, exceeding the control (1.08 g L^{-1}). The high biomass was likely due to enhanced NH_4^+-N utilisation, highlighting ADE's potential for mass culture. However, pH-10-treated ADE increased phycocyanin content by 45% relative to the control, possibly due to enhanced trace-element availability. Among the dilution treatments, the 4-fold dilution achieved the highest biomass yield (1.42 g L^{-1}) and productivity (0.26 g L^{-1} d^{-1}). In contrast, undiluted ADE inhibited the growth of *L. fusiformis* due to excessive ammonia. The 6-fold ADE dilution, on the other hand, showed the highest specific growth rate (0.25 d^{-1}); however, the growth declined after the exponential phase, possibly because of nutrient limitation. The 4-fold ADE dilution in this study supported sustained growth and prolonged productivity of *L. fusiformis* by maintaining sufficient nutrient availability and mitigating ammonia inhibition under alkaline conditions, where inhibition would typically be expected. These results demonstrate that water hyacinth-derived ADE can replace costly nitrogen sources and enable sustainable mass production, thereby enhancing the economic feasibility of large-scale production through optimised pretreatment.

Keywords: Anaerobic digestion effluent, Ammonium nitrogen, Limnospira fusiformis, Pretreatment

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OPTIMIZATION OF MAGNETIC HARVESTING OF FRESHWATER AND MARINE MICROALGAE USING CHITOSAN-COATED MAGNETITE NANOPARTICLES

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Microalgae are promising platforms for sustainable photobiorefineries. However, biomass harvesting remains a major economic bottleneck, accounting for up to 30% of total production costs due to small cell size (<30 μm) and stable suspension in culture media. This study systematically optimizes magnetic harvesting for freshwater strains *Limnospira fusiformis* (LA-08 and LC-30) and *Chlorella sorokiniana*, and the marine strain *Tetraselmis suecica*, using chitosan-coated magnetite nanoparticles (Fe_3O_4). Magnetite nanoparticles (128 ± 22 nm) were synthesized via co-precipitation and coated with chitosan to enhance electrostatic interactions, increasing the zeta potential from +20.3 mV to +44.4 mV. Harvesting performance was evaluated across a pH range of 2–12 and nanoparticle concentrations of 1–5.5 g g^{-1} (MNPs/dry cell biomass). The coated nanoparticles were positively charged below pH 7 and negatively charged above pH 7. Most microalgae exhibited a negative surface charge across the tested pH range, except *T. suecica*, which shifted to a positive charge under highly alkaline conditions. Harvesting efficiency was strongly pH-dependent*: C. sorokiniana* and *L. fusiformis* achieved up to 99% recovery at acidic pH (2–5), whereas *T. suecica* reached 97% under alkaline conditions. The optimal nanoparticle concentration ranged from 2.0–3.0 g g^{-1}, with rapid separation achieved within 1 min for freshwater strains and 3 min for marine *T. suecica*. The coated nanoparticles maintained high harvesting efficiency for up to four reuse cycles. These findings demonstrate that surface-modified magnetite enables fast, pH-tunable, and efficient, with reusable nanoparticles, providing a cost-efficient and scalable strategy for downstream processing in integrated microalgal photobiorefinery systems.

Keywords: Harvesting efficiency, Magnetic nanoparticle, Microalgae, pH, Zeta potential

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VALORIZATION OF CHEESE WHEY THROUGH STEADY CULTIVATION OF PROTEIN-RICH MICROALGAE

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This study evaluated the stability of protein-rich microalgae production using digested cheese whey (DCW) as source of nutrients. Cheese whey is the main byproduct of cheese production. It can be anaerobically digested to remove organic matter and convert it into biogas, while the digested effluent retains substantial nutrient loads, requiring further treatment or valorization. Although microalgae-based technologies have proven effective for the bioremediation of DCW, the stability of marine microalgae cultivation has not yet been thoroughly investigated. This study focused on assessing the stability of microalgae cultures where DCW was used as an alternative nutrient source throughout successive cultivation cycles of two microalgae with potential food and feed applications: *Nannochloropsis gaditana* and *Tetraselmis chuii*. Experiments were conducted under three culture conditions: synthetic medium (control), DCW as sole nutrient source, and a combination of both. At the end of each cycle (6-7 days), a portion of the culture was harvested and the remaining was used as inoculum for the subsequent cycle, with the corresponding addition of culture medium to restore the stablished initial conditions.

N. gaditana demonstrated stable performance for at least 24 days. Across all cycles, biomass production and nitrogen removal efficiency averaged 48% and 49% in DCW, and 73% and 81% in mixed medium, respectively, while biomass protein content did not vary with culture medium. *T. chuii* cultures were maintained for 49 days, and results varied depending on culture medium. Mixed medium cultures maintained consistently high nitrogen removal efficiencies, generally above 92%, and average biomass growth of 155% per cycle, indicating robust stability over 49 days. In contrast when DCW was used as the sole nutrient source *T. chuii* exhibited progressive deterioration, with nitrogen removal efficiency declining from 80% to below 45%, and mean biomass growth declined from 177% to 84%. Overall, the results demonstrate that DCW can totally or partially replace synthetic culture media across several cultivation cycles.

Keywords: Photorefineries, microalgae, cheese whey, nutrients recovery, single-cell protein.

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OLIVE-MILL WASTEWATER PARTICLES AS EFFECTIVE BOOSTERS OF *ARTHROSPIRA PLATENSIS* METABOLISM DURING PHOTOSYNTHETIC BIOGAS UPGRADING

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The addition of biostimulant particles (BPs) derived from olive-mill wastewater (OMWW) has emerged has a practical technique to improve microalgae performance during photosynthetic biogas upgrading (PBU) and has motivated the use of pigment-producing microalgae to improve the competitiveness of the process. However, a clear understanding of the interaction between the particles and microalgae is necessary to design proper materials that maximize both PBU overall performance and pigment production.

In this study, BPs with varied iron content (0,7 and 15% dw) were synthesized and added to *A. platensis* cultures during batch PBU tests (400 μmol $m^{-2}s^{-1}$, 250 rpm and 25 ºC) at a concentration of 100 mg L^{-1}. The results showed that the three biostimulant BPs boosted *A. platensis* growth rate and phycocyanin (C-PC) content, regardless of the iron content (**Fig.1**). Transcriptomic analysis revealed that the BPs improved PSII recovery and nutrient uptake, particularly extracellular nitrate, phosphate and sulphate suggesting that supplemented cultures became more energy-efficient. However, the results demonstrate that iron was not necessary to boost *A. platensis* performance in fact, cultures supplemented with non-iron-containing BPs presented a C-PC content of 325 mg g^{-1}_{dw}, which was 48% higher than cultures without BP supplementation.

Further light-stress conditions (800 μmol $m^{-2}s^{-1}$) were induced to validate non-iron-containin BPs effect and, supplemented cultures presented a specific growth rate 15% higher than the cultures without BP supplementation and a C-PC content similar to the cultures cultivated under 400 μmol $m^{-2}s^{-1}$. Transcriptomic analysis revealed that the BPs reduced stress in the high light incubated cultures and thereby, were able to mantained a consistent growth. This work demonstrates that BPs supplementation stands as a practical technique to boost *A. platensis* metabolism and the overall techno-economic feasibility of PBU.

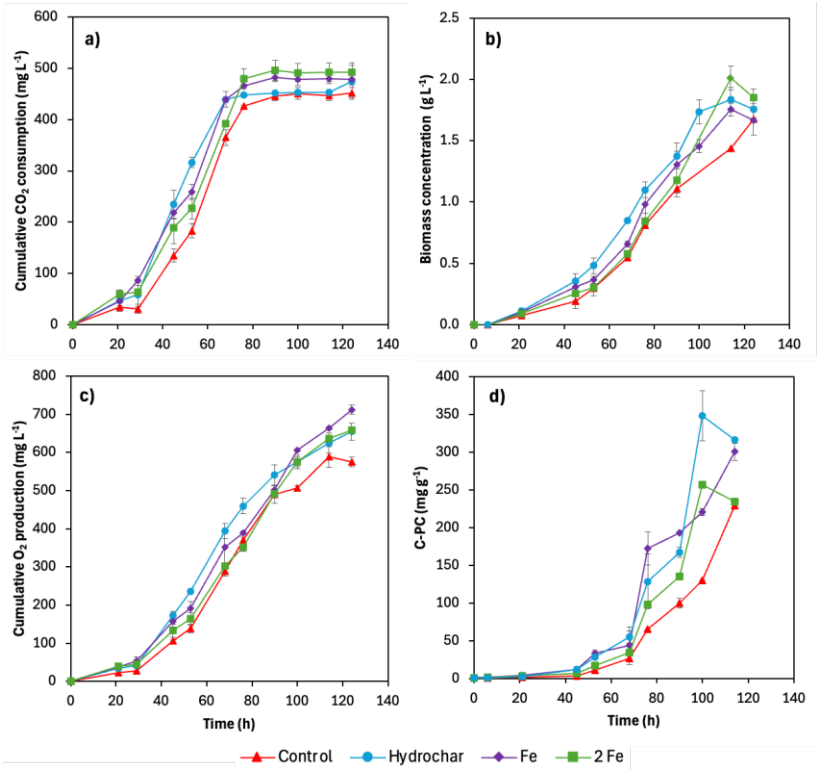


Fig. 1 Time course of the a) cumulative CO_2 consumption (mg L^{-1}); b) biomass concentration (g L^{-1}); c) cumulative O_2 production (mg L^{-1}) and d) cumulative phycocyanin production (C-PC, mg L^{-1}) of *A. platensis* cultures without BPs (control), and supplemented with 100 mg L^{-1} of hydrochar, Fe, and 2Fe BPs. Note BPs: biostimulant particles, hydrochar: nanoparticles without iron; Fe: nanoparticles containing 7% iron; and 2 Fe: nanoparticles containing 15% iron.

Keywords: antioxidant, biostimulant, extracellular interaction, nutrient uptake

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ASSESSING THE BIODIGESTIBILITY OF DIGESTATE-GROWN MICROALGAL BIOMASS

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Microalgae biomass is emerging as a potential solution for the agricultural and livestock industry by converting biogas by-products (digestate and CO_2) into high-value protein. Algal biomass grown in digestate as a nutrient source shows high protein content (around 35-45%) and a suitable amino acid profile for animal feed, surpassing soybean and other conventional sources in quality. However, larger-scale applications are still restricted by several technical and regulatory barriers regarding the use of digestate. In addition, the recalcitrant nature of green microalgae cell walls limits the accessibility of intracellular proteins, while growth conditions (digestate vs. mineral medium) and post-harvesting processes may also influence the biomass digestibility. In the present study, a methodology based on *in vitro* digestibility tests was optimized. This methodology simulates monogastric animal digestion, consisting of a two-step process representing the gastric and intestinal phases. The analyzed biomass was obtained from the cultivation of *Parachlorella kessleri* and *Desmodesmus denticulatus* in pilot photobioreactors (PBRs) using both mineral medium (modified BBM) and diluted digestate (2-4%). Results reveal that microalgae biomass is more digestible before drying and milling (60% against 36% after processing); however, these processes are desirable to ensure product stability. Consequently, different post-treatments were applied to dried and milled biomass to simulate industrial feed processing. Temperature significantly improved protein solubility (via autoclave, water bath, and heated ultrasound), increasing it by 30%-60% compared to the untreated milled biomass, especially for biomass grown in digestate (50-60%). Soft alkaline treatments (50 mM KOH) also resulted in higher biodigestibility across all samples (51-57%); however, combined pretreatments (temperature + KOH) resulted in 32-48%. Under the previous conditions, the same biodigestibility levels as pig feed references (54 ± 5 %) were achieved. Future research will focus on analyzing protein characteristics (peptide size and amino acid profile) to better understand the suitability of digestate-grown algae as a feed ingredient and how to enhance post-solubilization absorption. Moreover, these pretreatments could serve as additional stabilisation and sterilisation steps, ensuring high biomass quality for the feed industry.

Keywords: Parachlorella kessleri, Alternative Proteins, In vitro digestion, Feed

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Section 3.
PHOTOSYNTHETIC BIOTECHNOLOGY FOR WATER AND AIR TREATMENT

FROM GREENHOUSE GAS TO GOODS: PPB TREATING CARBON MONOXIDE & SYNTHESIS GAS

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The European Union (EU) must curtail industrial greenhouse gas (GHG) emissions and find alternative energy sources (e.g., bio-hydrogen) to transform into a decarbonised, green economy. Purple phototrophic bacteria (PPB) provide a solution to the issues of industrial pollution (liquid and gas) and resource security, while producing valuable products, e.g., high-protein biomass, H_2 fuel, and pigments. Through genome mining, the authors acquired 8 PPB species (*M. purpuratum*, *R.rubrum* 107, *R. rubrum* 467, *Rhodopseudomonas* sp., *R. euryhalinum*, *R. robiginosum*, *R. strictum*, *R. marina*) with genomic potential to utilise CO_2, CO and produce valuable products. The end goal was to generate sufficient robust data with which to generate and validate a world-first PPB mathematical model describing syngas/CO fixation. Strains grew in batch conditions using a) standard DSMZ growth medium (photoheterotrophy), b) standard DSMZ growth medium with CO/N_2 headspace to test for CO-sensitivity, c) CO as the sole carbon and energy source (carboxydotrophy; CO/N_2 headspaces at 10, 15, 20, and 40%-CO) and d) syngas as the sole carbon and energy source (carboxydotrophy/photoautotrophy). *R. rubrum* 467 was the sole capable CO-metaboliser. Illuminated batch experiments with *R. rubrum* revealed strong CO-dependent gas conversion kinetics but limited biomass formation under CO/N_2 atmospheres. Complete CO removal occurred within 7-20 days, with faster depletion at 15% CO. Maximum H_2 evolved at 50 $mmol_{H2}$ g_{PPB}^{-1} d^{-1}. Maximum headspace H_2 concentrations increased with CO fraction, reaching 9 ± 2 % at 10% CO and 17 ± 1.8 % at 20% CO, while CO_2 accumulated at 4.2 ± 0.88 and 9 ± 1.57 %, respectively (due to the higher solubility/k_La of CO_2). Biomass growth remained slow in all CO/N_2 bottles, increasing only from 0.04 g L^{-1} to end up stabilising at 0.104 ± 0.042 g L^{-1} over four cycles. Carbon mass balances showed consistent CO oxidation, with yields of 0.8-0.9 $molCO_2$ $molCO^{-1}$ and bio-hydrogen yields of 0.8-0.9 $molH_2$ $molCO^{-1}$, confirming water-gas shift (WGS)-dominated activity, with the remaining carbon being utilised for cellular growth. In contrast, syngas supported rapid and sustained growth; biomass increased from 0.03 to 1.2 g L^{-1}. Complete syngas consumption repeatedly occurred within 5 days, following sequential CO removal and subsequent simultaneous CO, CO_2, and H_2 uptake, consistent with enhanced photoautotrophic metabolism. In summary, biomass yields were lower under the CO-only conditions, indicating redox-driven metabolism dominated by WGS. In contrast, *R. rubrum's* higher biomass under syngas is consistent with enhanced CO_2 fixation via photoautotrophy. This work has widened the understanding of PPB's suitability and capability to treat toxic gases such as CO and GHGs like CO_2, and its usefulness as a bio-energy producer. These experimental rates, yields, and stoichiometries provide quantitative foundation for mathematical modelling of phototrophic syngas fermentation and bio-hydrogen production via CO gas with the PPB *R. rubrum*.

Keywords: Bio-hydrogen, Carbon monoxide, Purple phototrophic bacteria, Added value products

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DATA-DRIVEN OPTIMIZATION AND ADAPTIVE CONTROL OF PHOTOSYNTHETIC BACTERIA CULTIVATION IN OUTDOOR PHOTOBIOREACTORS USING MACHINE LEARNING

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Photosynthetic bacteria (PSB) offer a promising route for wastewater resource recovery because they can simultaneously remove organic pollutants and convert wastewater nutrients into value-added biomass. However, PSB cultivation under natural outdoor conditions is difficult to optimize using conventional empirical methods, while machine learning provides a powerful tool to reveal nonlinear environmental responses and guide intelligent cultivation control.

In this study, a data-driven prediction framework was developed by integrating literature-derived PSB cultivation data with five-month outdoor pilot-scale operation data from two tubular photobioreactors with diameters of 110 mm and 75 mm. A BiLSTM–Transformer hybrid model was developed to predict PSB growth and support adaptive regulation in outdoor photobioreactors. The model input variables included temperature, light intensity, light quality, photoperiod, dissolved oxygen, pH, C/N ratio, inoculation ratio and wastewater characteristics, while the main outputs included biomass production and COD removal efficiency. In addition, SHAP analysis and interaction interpretation were used to identify key environmental drivers and effective operating ranges.

The R^2 value of the model reaches 0.97, indicating good predictive performance. Concurrently, the results of this model differ significantly from those of machine learning models built using laboratory data, particularly in terms of feature importance. SHAP-based feature importance analysis showed that temperature was the most influential factor affecting PSB growth, contributing 33.7% to model interpretation, followed by light intensity with a contribution of 29.4%. This result indicates that temperature exceeded light intensity as the dominant environmental driver under outdoor cultivation conditions. Interaction analysis between temperature and light intensity further revealed two critical temperature thresholds at 17.6 °C and 29.3 °C. When the temperature was below 17.6 °C, changes in light intensity could not compensate for the inhibitory effect of low temperature. When the temperature was above 29.3 °C, adjustment of light intensity could not effectively alleviate high-temperature inhibition. Furthermore, within the temperature threshold, light intensities ranging from 0 to 31,495 Lx have been shown to have a positive effect on PSB; however, when light intensity exceeds 31,495 Lx, it has been demonstrated to inhibit PSB growth. These results suggest that outdoor PSB cultivation should prioritize temperature-centered regulation rather than relying only on light management.

The cultivation substrate also strongly affected system performance. Compared with artificial wastewater, anaerobic digestion slurry increased PSB growth efficiency by 26.1% (biomass increased from 999.41 mg/L to 1260.91 mg/L) and improved COD removal efficiency by 9.0% (increased from 78.26% to 85.30%), demonstrating its suitability as a nutrient-rich medium for PSB-based wastewater resource recovery. Furthermore, the trained model was successfully applied to real-time adaptive control and online yield prediction, demonstrating excellent performance. Overall, this study demonstrates that machine learning can identify key environmental constraints, quantify interactions among characteristics and enable fast decision-

making for outdoor PSB cultivation, providing a practical basis for intelligent photobioreactor operation and scale-up application.

Keywords: photosynthetic bacteria; machine learning; BiLSTM–Transformer; photobioreactor; adaptive control; biomass prediction; wastewater resource recovery

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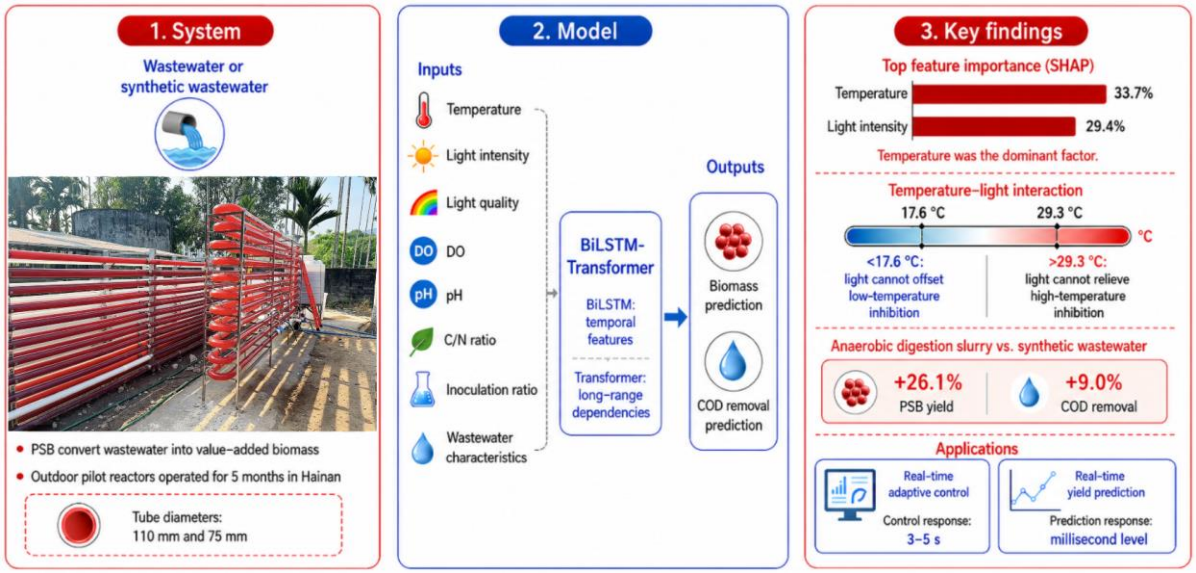


Fig. 1: *High-efficiency cultivation of photosynthetic bacteria guided by machine learning.*

DEGRADATION OF EMERGING MICROCONTAMINANTS BASED ON PHOTOSYNTHETIC SYSTEMS

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The widespread occurrence of emerging micropollutants, including pharmaceuticals, hormones, and personal care products, in wastewater has become a major environmental concern, as conventional treatment systems are not designed for their efficient removal. In this context, phototrophic-based processes have gained increasing attention due to their ability to integrate multiple removal pathways, such as biodegradation, photodegradation, and biosorption. This study comparatively evaluated micropollutant removal in two photobioreactors: a microalgae-bacteria consortium (AB) and a mixed culture of purple phototrophic bacteria (PPB), with particular emphasis on the effects of hydraulic retention time (HRT) and carbon-based nanoparticle addition. Two continuous photobioreactors (3L) were operated using synthetic wastewater with a concentration of dissolved total organic carbon (TOC) of ~375 $mg \cdot L^{-1}$ under four sequential stages: biomass acclimation, micropollutant loading (200 $\mu g \cdot L^{-1}$ of sulfamethoxazole, trimethoprim, diclofenac, methylparaben, and estradiol), HRT reduction (6 to 4 days), and nanoparticle supplementation (2 $mL \cdot L^{-1}$). The PPB system achieved TOC removal efficiencies >90% while AB treatment 80-90%. Total nitrogen removal showed high variability (60-90%) with no statistically significant differences between the systems in the aforementioned phases, while phosphorus removal was higher in PPB (>60%) than in AB (<50%). PPB showed superior removal efficiencies for trimethoprim, sulfamethoxazole, and methylparaben (>70%), while AB achieved moderate removal of 50-75% for the latter two, and <30% for trimethoprim. Diclofenac and estradiol showed lower removal efficiencies (<60%) in both systems, confirming their persistence. A decrease on the HRT resulted in up to a 20% reduction in the removal of diclofenac and sulfamethoxazole in both systems. However, the removal efficiency of estradiol increases (>15%), which may be associated with an increased metabolic activity of the microorganisms. The addition of carbon-based nanoparticles increased the removal of the most recalcitrant compounds by 10–25%, likely due to improved redox mediation and stimulation of microbial activity. Overall, both systems demonstrated high robustness and efficiency in the simultaneous removal of organic matter and micropollutants, reinforcing the potential of photosynthetic systems as sustainable platforms for advanced wastewater treatment.

Keywords: Emerging micropollutants, Microalgae; Photobioreactors, Purple phototrophic bacteria

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BIOLOGICAL NITROGEN REMOVAL FROM AN ANAEROBIC DIGESTATE LIQUID FRACTION APPLYING CONSORTIA OF MICROALGAE AND NITRIFYING BACTERIA UNDER NATURAL IRRADIATION IN GUANAJUATO, MÉXICO

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The main objective of this study was evaluating the performance between microalgae and nitrifying bacteria on the nitrogen and nutrient removal from liquid fraction of anaerobic digestate and under environmental conditions in Guanajuato, México, one of the most important is sun light radiation. The microalgae- nitrifying bacteria consortium can enhance the removal of nitrogen and other nutrients from the liquid fraction of anaerobic digestate from the slaughterhouse wastewater through the synergy between the two microorganisms improving the effluent quality. The pilot raceway reactor operated under an average light irradiance of 450 µmol photons $m^{-2} \cdot s$. The raceway used is a 30 L reactor made by glass fiber with a stirring system. The continuous operation of the raceway was along 77 days with 40 days under a HRT of 7 days (phase 1), 23 days with HRT of 5 days (phase 2) and 14 days with HRT pf 3 days (phase). On this outdoor experimental period were evaluated mainly the nitrogen and nutrients removal from liquid digestate by the microalgae-nitrifying bacteria dispersion culture and the predominance of microalgae and bacteria genus on each experimental phase. The ammoniacal nitrogen removal from an anaerobic digestate of a slaughterhouse located in Guanajuato, México using a consortium of nitrifying bacteria - microalgae get a 70.24% removal of NH_4^+ in the dispersion system with 7 days of Hydraulic Retention time (HRT), 70.76% at 5 days of HRT and 66.60% with HRT= 3 days. The average removal for phosphates was above 50% for each experimental phase. The effluent from the microalgae- nitrifying bacteria consortium treatment could be destinated to irrigation zones in Guanajuato, because Mexican Normativity Mention as “No apply” the NT and TP concentration for irrigation, therefore this water could be reused, and de Nitrogen and Phosphorus remained would be part as a soil enrichment.

Keywords: anaerobic digestate, microalgae, nitrifying bacteria, symbiosis

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CLEAN WATER, RICH SOILS, PIG DIET: A CIRCULAR MICROALGAE PATHWAY FOR PIG FARMS

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Pig farming is a major source of animal protein worldwide generates large volumes of piggery wastewater (PWW) rich in organic matter and nutrients. Its dark color, high turbidity and toxic concentration of ammonia make treatment challenging.

This work evaluated an integrated Vertical Flow Constructed Wetland-Microbial Fuel Cell-Microalgal Photobioreactor (VFCW-MFC-PBR) system for simultaneous wastewater treatment, bioelectricity generation, and microalgal biomass production within the framework of the Portugal–India WCAlgaeKIT project, aimed at sustainable solution for small rural pig farm in India.

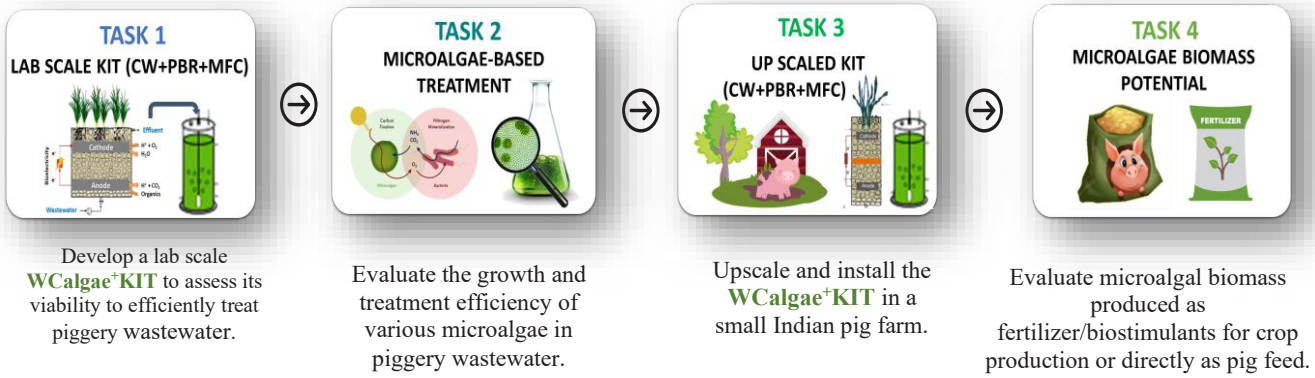
The VFCW pretreatment, composed of a two-stage system with graded gravel media (3 layers), *Canna indica* plants, passive aeration, significantly improve the PWW quality and enabled the cultivation of *Tetradesmus obliquus* microalga, enhancing early growth kinetics by reducing turbidity and inhibitory compounds. The MFC provided additional functionality through bioelectricity generation while maintaining effluent compatibility for algal cultivation.

At pilot scale (4 L d^{-1}), the system achieved removals of ~90% for chemical oxygen demand (COD), total organic carbon (TOC), and phosphate, 87% for total ammoniacal nitrogen (TAN), and 67% for total nitrogen (TN). Hydraulic saturation in VFCW-MFC proved essential for establishing stable redox gradients. The best distance between electrodes was 60-20 cm from the top achieving a maximum bioelectricity generation of up to 539 mV (CE ~18–25%). *T. obliquus* cultures achieved biomass yields of 1.1-1.3 g L^{-1}, being promising as biofertilizers/biostimulants. Previous trials have shown that *T. obliquus* grown in PWW stimulated a 20-105% increase in germination index of wheat, while the nutrients still present in the respective treated PWW sustained wheat growth [1]. Techno-economic analysis indicated that the VFCW-MFC system is cost-effective and energy-efficient, with a treatment cost of 0.41 m^{-3} and energy consumption of 0.16 kWh m^{-3}. The potential of microalgal biomass for agriculture and pig feed will be further assessed, as well, as the overall tecno-economical and LCA analysis.

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Constructed Wetlands (CW) + Microbial Fuel Cell (MFC)+ Microalgae Photobioreactor (PBR)



Develop a lab scale **WCalgae⁺KIT** to assess its viability to efficiently treat piggery wastewater.

Evaluate the growth and treatment efficiency of various microalgae in piggery wastewater.

Upscale and install the **WCalgae⁺KIT** in a small Indian pig farm.

Evaluate microalgal biomass produced as fertilizer/biostimulants for crop production or directly as pig feed.

Reference:

1. Ferreira, A.; Figueiredo, D.; Ferreira, F.; Marujo, A.; Bastos, C.R.V.; Martin-Atanes, G.; Ribeiro, B.; Štěrbová, K.; Marques-dos-Santos, C.; Acién, F.G.; et al. From Piggery Wastewater to Wheat Using Microalgae towards Zero Waste. *Algal Res.* **2023**, *72*, 103153, doi:10.1016/j.algal.2023.103153.

CULTIVATION AND SELECTION OF ANOXYGENIC PURPLE SULFUR BACTERIA FROM WASTEWATER FOR H_2S REMOVAL AND CO_2 SEQUESTRATION

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Unique metabolism of anoxygenic phototrophic sulfur bacteria (APSB) made them promising candidates for processes with simultaneous H_2S removal and CO_2 sequestration by biomass production and biologically induced precipitation. These microorganisms use reduced sulfur compounds (e.g., H_2S) as electron donors and CO_2 as a carbon source in anoxic and luminous (infrared) environment. Their viability in contaminated environment, such as wastewater, makes them applicable for bioremediation and removal of H_2S and CO_2 from gasses, such as treatment and/or upgrading of biogas or syngas. Here, APSB cultivation and selection from wastewater was achieved, while also gaining deeper insight on sulfur metabolism.

To understand APSB's role in H_2S and CO_2 removal and the impact of wastewater consortia on its metabolism, three experimental setups were employed: i) sequential batch experiments with varying *Allochromatium vinosum* and wastewater ratios (1:0, 0.8:0.2, 0.5:0.5 v:v), ii) a fed-batch bioreactor with continuous sulfide supply, and iii) a subsequent triplicate bioreactors with only wastewater as inoculum for selection experiments. Inorganic synthetic medium was used across the experiments to ensure photoautotrophic metabolism. The chemical environment (pH, inorganic carbon, chemical oxygen demand, cations, and anions) was monitored alongside microscopy, Fluorescence in situ hybridization (FISH), qPCR, and 16S rRNA sequencing.

Sulfur oxidation and CO_2 sequestration, indicative of APSB activity, were observed across all experiments. Microscopy, qPCR, and FISH confirmed APSB presence and a stable consortium in sequential batches, particularly at higher wastewater ratios, and detected APSB in the initial wastewater. Consistent sulfur oxidation and COD removal rates across sequential batch and fed-batch experiments suggested robust APSB behaviour in both pure and mixed cultures, and even showed APSB enrichment in the latter. Despite an initial adaptation delay, the selection experiment yielded a diverse and promising consortium, confirming APSB behaviour remains viable and competitive in non-sterile, contaminated environments.

Keywords: Carbon Dioxide, Sulfide Treatment, Anoxygenic Phototrophic Sulfur Bacteria (APSB)

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INSIGHTS INTO THE IMMOBILIZATION BEHAVIOR OF MICROALGAL-BACTERIAL CONSORTIA (MBC) UNDER VARYING AERATION AND MIXING CONDITIONS

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Microalgal-bacterial consortium (MBC) seems promising compared to sole actors of the consortium in many different aspects such as treatment of municipal wastewaters and some industrial (eg. food and piggery) wastewaters, lower aeration/energy need, better harvesting characteristics, CO_2 sequestration and nutrient, COD and heavy metal removal capacity. In this study, it is aimed to investigate the effect of aeration and mixing on immobilization of MBC and removal performances. Thus, three reactors (R1-R3) inoculated with enriched C.*vulgaris* and activated sludge were operated at different aeration and mixing conditions. Both aeration and mixing were applied to R1, only aeration was applied to R2, and only mixing was applied to R3. Reactors were opered at 24h illumination, pH values of 6.5-8.0, 25 ± 4°C, mixing (if any) at 180 rpm, and aeration (if any) at 0.4 L/min (0.625 vvm). Polypropylene media (effective surface area of 500 m^2/m^3) was used as biocarrier. Modified Bold's basal medium with 66-270 mg/L NH_4-N, 8-34 mg/L PO_4-P, and 1030-3653 mg/L glucose (1100-3900 mg/L COD) were fed every 48 hours with a volume exchange ratio of 12.5% (HRT of 16 days).

Visual and microscopic observations revealed that biocarriers placed in the reactors were immobilized with microalgae, only after a couple of days. After more than 100 days of operation, the biocarriers in R1 and R2 (both continuous aerated) retained their green coloration with dark green, granule-like structures within biocarriers. The suspension in both R1 and R2 exhibited a brownish-green coloration, suggesting bacterial existence in bulk liquid. Kinetic and metagenomic investigations conducted to elucidate the removal mechanisms revealed a clear bacterial dominance, attributable to nitrification–denitrification being the primary nitrogen removal pathway. In contrast, R3, subjected to only mixing (no aeration), exhibited a distinct immobilization period unlike R1 and R2. The color of suspension and the biocarriers of R3 shifted to brownish red and TAN (up to 116 mg/L) accumulated which was further recovered. This coloration was primarily attributed to stress-induced carotenoid production by microalgae. Metagenomic and microscopic analyses confirmed the dominance of *Coelastrella saipanensis*, a species known for synthesizing astaxanthin under adverse conditions. The lack of aeration and high nutrient accumulation appeared to increase the susceptibility of the MBC. Notably, *Rhodopseudomonas sp*. constituted approximately one-third of the bacterial population, further contributing to the observed color change. These results indicate that the synergistic metabolic activity of *Coelastrella saipanensis* and *Rhodopseudomonas sp.* facilitates the observed color shift under high nutrient-induced stress for R3.

This study revealed that aeration+mixing, in particular aeration, seems to be more critical at high nutrient loadings, for improved nutrient removal and a more resistant MBC development. Mixing, the main difference between R1 and R2, significantly shortens the acclimation time. The absence of aeration, coupled with high nutrient loading, triggered a significant microbial shift

within the MBC (R3). These results suggest that controlled mixing without aeration can be effectively utilized to induce stress-driven pigment accumulation for extraction purposes.

Keywords: MBC, immobilization, nutrient removal, functionality, aeration and mixing effect

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OPTIMISATION AND SEMICONTINUOUS OPERATION OF SCENEDESMUS SP. FOR THE TREATMENT OF RAW BREWERY WASTEWATER

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Microalgal systems in the brewing industry are traditionally relegated to a secondary 'polishing' role following anaerobic digestion to address residual nutrient loads (Amenorfenyo et al., 2019 https://doi.org/10.3390/ijerph16111910). However, this sequential approach fails to utilise the rich organic carbon source present in raw effluents. Our study diverges from this convention by demonstrating that raw brewery wastewater can serve as a direct primary growth medium, utilising the organic load to drive mixotrophic productivity and achieve superior nitrogen remediation in a single biological step.
Initial batch experiments were conducted in three 4.5 L replicates over seven days to monitor microalgae-bacteria biomass growth and nutrient depletion. The resulting data were fitted to a Verhulst logistic model to predict volatile suspended solids (VSS) dynamics and determine the optimal hydraulic retention time (HRT). While the model predicted an optimal HRT of 3.2 days, a conservative HRT of 4 days was selected for the semicontinuous phase to ensure culture stability.
In the semicontinuous stage, four 4.5 L replicates were operated with daily harvesting and feeding. The system maintained stable performance for 24 days, achieving a steady-state VSS concentration of 0.76 g/L before a subsequent decline in cell concentration. Nutrient removal efficiencies were significant: Total Nitrogen (TN) was reduced by 77%, with ammonium (NH_4^+) removal reaching nearly 100%. Total Phosphorus (TP) and Total Organic Carbon (TOC) removal efficiencies were 48% and 34%, respectively. These results demonstrate that raw brewery wastewater can support robust microalgal-bacterial consortia, providing an effective one-step biological treatment solution. The application of the Verhulst model proved to be a reliable tool for scaling batch kinetics to continuous systems, highlighting the potential for industrial-scale wastewater valorisation.

Keywords: Nutrient bioremediation, Raw brewery wastewater, Scenedesmus sp., Semicontinuous cultivation, Verhulst model.

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FOOD INDUSTRY EFFLUENTS AS A CULTURE MEDIUM FOR CYANOBACTERIA

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Wastewater treatment by microalgae has been extensively investigated. This process not only offers a cheaper medium for the culture, but also reduces water pollution, fixes CO_2 and can lead to the production of value-added products from microalgae. Industrial effluents pose special interest as they can legally be considered as by-products rather than wastes, contrary to urban wastewater (Directive 2008/98/EC of the European Parliament). In this study, the growth of the cyanobacterium in effluents from plant based beverages production is assessed, as a promising effluent for the production of cyanobacterial biomass.

An initial screening was carried out to select the most appropriate effluent and cyanobacterium strain. To this end, integrated effluent samples were characterized, and different cyanobacteria were grown in 0.5 L flasks, operated in batch mode for 7 days. Five effluents from different points of the treatment plant were used, selecting the outlet effluent from the UASBR as the most suitable.

Next, the selected strain (*A. platensis*) was grown in 4L batch photobioreactors. Experimental data analysis of the growth and nutrient consumption, by fitting the models BIO_ALGAE and PHB (Solimero, A. et al., Sci. Total Environ, 601-602, 646-657;Rueda, E., Garcia, J. (2021) Sci. Total Environ., 800, 149561) suggested an optimum hydraulic retention time (HRT) of 5 and 8 days. Both HRT were explored in semi-continuous mode over a period corresponding to 3 HRT, monitoring the biomass quantity and quality, as well as the nutrient removal. With a HRT of 8 days, a constant biomass production around 0.5 g/L was obtained, in agreement with the model prediction, which corresponded to an increase of 150% with respect to the initial biomass. For the HRT of 5 days, the final biomass concentration was 0.24 g/L, meaning an increase of 100% with respect to the initial biomass concentration.

Keywords: Bioproducts, Circular bioeconomy, Food industry, Microalgae, Wastewater

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MINIMISING AERATION REQUIREMENTS IN A PHOTOGRANULAR SYSTEM FOR THE TREATMENT OF WASTEWATER AT SHORT HRT

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The synergy between microalgae and bacteria within photogranules (PGs) enables reduced energy consumption (requiring minimal or even no aeration) and a smaller footprint compared to conventional microalgae systems. PGs have been applied to wastewater treatment, achieving efficient pollutant removal without aeration, but their application has been restricted to low organic and nitrogen loads or long hydraulic retention times (HRT). The present study aimed to evaluate the limits of aeration reduction in photogranular systems under conditions typically applicable to aerobic granular sludge (AGS) (high carbon and nitrogen loads at short HRT).

Two column-type photobioreactors were operated. R1 was run without aeration for 74 days, mixture provided by a mechanical stirrer (50 rpm). R2 operated at different aeration flow rates (L/(L·min)) in six stages (S.) for 228 days: 1.65 (S.I), 1.24 (S.II), 0.82 (S.III), 0.41 (S.IV), and 0.41 and 0 (S.V and S.VI, respectively), adding a mechanical stirrer in these last two stages. Both reactors were continuously illuminated and operated as sequencing batch reactors in 3-hour cycles with an HRT of 0.24 days. The inoculum was AGS and microalgae. The feeding was synthetic, with 500 and 750 mg COD/L for R1 and R2, respectively. The feeding C:N:P ratio was of 100:20:4 for R1 and of 150:20:4 for R2.

The results indicate that R1 failed to achieve significant pollutant removal, with only 14% and 39% removal of ammonia and carbon, respectively. There was neither nitrite nor nitrate in the effluent. Nevertheless, the PGs remained well-formed. R2 maintained stable performance with ammonia and carbon removals above 90 % down to an aeration rate of 0.82 L/(L·min) (S.I to S.III), with nitrite being the unique form of nitrogen in the effluent. At lower aeration rates, S.IV and S.V, consumption kinetics slowed, and the cycle was not long enough to ensure the required nitrogen removal. Based on the kinetics, an aeration rate of 0.41 L/(L·min) together with mechanical stirring would be appropriate if the cycle length was 4 hours. In S.VI, microalgae could not meet the bacterial oxygen demand, likely due to the high mean PG diameter (4.7 mm). Further research is needed to evaluate the performance of these PG reactors by controlling the solid retention time to maintain an optimal granule diameter for in situ oxygen production.

Keywords: Aeration-free, Aerobic granular sludge, Microalgal-bacterial granules, Phototrophic granulation, Wastewater treatment.

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DEVELOPING A MICROALGAL-BACTERIAL CONSORTIUM FOR THE REMOVAL OF POLLUTANTS FROM AGRO-INDUSTRIAL WASTEWATER UNDER SEASONAL TEMPERATURE CONDITIONS

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The generation of wastewater from food industries constitutes both an environmental challenge and an opportunity to produce high-value bioproducts. Currently, effluents from food processing industries are mainly treated using aerobic activated sludge systems, which are associated with high energy demand, losses of carbon and valuable nutrients, and a significant carbon footprint. In the context of circular economy and decarbonization strategies, microalgae have emerged as a promising strategy for converting the residual carbon, nitrogen and phosphorus present in these wastewaters into algal biomass, thereby enabling the development of new value chains in the agro-industrial sector. Moreover, microalgal growth can be enhanced through synergistic interactions with growth-promoting bacteria. Thus, the aim of this work was to develop a microalgal-bacterial consortium based on growth performance and the removal efficiencies of total organic carbon (TOC) and total nitrogen (TN), under temperature conditions representative of seasonal variations in southern Spain (28 and 11 ºC). For this purpose, 20 microalgal strains and 6 plant growth-promoting bacterial strains were cultivated in vegetable-processing wastewater (VW) to evaluate their growth in this medium. Based on growth performance in microplate assays, five microalgal strains were selected: *Chlorella sorokiniana*, *Scenedesmus quadricauda*, *Coelastrella thermophila*, and two strains isolated from the wastewater (WW9 and WW10). Among these, *Scenedesmus quadricauda* and WW9 exhibited the highest performance in serum-bottle assays, considering both biomass production and TOC and TN removal. Regarding bacterial strains, *Azospirillum brasilense* and *Serratia liquefaciens* were selected based on their growth in VW. The resulting microalgal-bacterial consortia were evaluated, and the best-performing combination was subsequently cultivated in continuous photobioreactors to confirm its potential for efficient nutrient removal from VW.

Keywords: bacteria, circular economy, lettuce wastewater, microalgae

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MICROALGAL BIOFILMS TREATING LOW-STRENGTH WASTEWATER: INFLUENCE OF LIGHT INTENSITY AND SHORT RETENTION TIMES ON BIOMASS PRODUCTIVITY, TREATMENT PERFORMANCE AND BIODIESEL POTENTIAL

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The abstract should refer to the most relevant backgrounds, significant results and contribution conclusions. It should not exceed one A4 page in the given format and preferably should be written without any references. In an A4 sheet use a margin of 3 cm on the top, while 2.5 cm-s should be used on the right, left and bottom. Formulas should be centralized and numbered on the right. The file with the abstract should be named with the surname and first name of the corresponding author (for example: Surname_Firstname_Abstract).

Microalgal biofilm systems are increasingly regarded as an energy-efficient alternative for wastewater treatment because they reduce aeration demand and facilitate high-density biomass harvesting. Yet, under low-strength municipal wastewater conditions, the simultaneous achievement of high treatment efficiency and high biomass productivity remains constrained by the operational balance among light supply, biomass retention, and nutrient loading. In this study, a horizontal microalgal biofilm photobioreactor treating synthetic primary effluent was operated at short hydraulic retention times (HRT, 1–1.5 h), while solids retention time (SRT, 6–21 d) and light intensity (LI, 80–500 μmol m^{-2} s^{-1}) were systematically varied to identify conditions favoring either treatment performance or biomass valorization.

Across six operating conditions, decreasing SRT and increasing LI increased areal biomass productivity by nearly one order of magnitude, from 4.6 ± 1.6 to 45 ± 18 g m^{-2} d^{-1}, confirming that frequent harvesting and strong irradiance are key drivers of biofilm growth under high-rate operation. However, the highest biomass productivity did not coincide with the best treatment performance. Maximum COD and TN removals were achieved at an intermediate LI (240 μmol m^{-2} s^{-1}) combined with a short SRT (6 d), reaching 80 ± 4% COD and 87.1 ± 1.6% TN removal, even under the elevated loading rates associated with reduced HRT. In contrast, operation at 500 μmol m^{-2} s^{-1} led to lower COD and TN removals despite enhanced biomass production, suggesting that excessive irradiance shifted the system away from optimal treatment conditions. The appearance of yellowish-green pigmentation and gas bubble accumulation, together with the development of thicker and more heterogeneous biofilms, supports the interpretation that photostress and internal mass-transfer limitations became dominant under these conditions.

Phosphorus removal remained comparatively stable across all treatments (73.9 ± 1.1%; effluent < 1.2 mg P L^{-1}), indicating that P removal was less sensitive to the operational factors evaluated and may have been strongly influenced by physicochemical retention under persistently alkaline conditions (pH > 9). From a resource recovery perspective, high LI markedly increased FAME accumulation, from 3.42% to 19.69% of dry weight, while maintaining a profile dominated by C16–C18 fatty acids. These results highlight a fundamental trade-off in microalgal biofilm operation: conditions that maximize biomass and lipid productivity are not necessarily those that optimize wastewater treatment. Therefore, SRT, HRT, and LI should be regarded not simply as operating parameters but as control variables that determine whether the system is directed toward treatment efficiency or biomass valorization.

Keywords: low-strength wastewater, Fatty Acid methyl ester, nitrogen, biomass productivity

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Section 4.
TECHNOLOGIES FOR POST-TREATMENT, EXTRACTION AND PURIFICATION OF BIOMASS AND BIOPROCESS RESIDUAL STREAMS

WATER, ENERGY & OIL RECOVERY THROUGH A PALM OIL-BASED PHOTOBIOREFINERY APPROACH

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The global drive towards climate-neutral, resource-efficient industrial systems has put increasing pressure on agro-industrial sectors like the palm oil industry. Malaysian palm oil mills on average generate 2.5 – 3 tonnes of Palm Oil Mill Effluent (POME) and 1.1 – 1.5 tonnes of oil palm empty fruit bunch (OPEFB) per tonne of Crude Palm Oil (CPO) produced, with a typical Malaysian mill processing 100000 ton of Fresh Fruit Bunch per year. With the sector still reliant on fossil fuel/ or conventional biomass combustion, a transition to photobiorefineries offers ample opportunities to recover water, energy, carbon and lipids.

In this research compilation, the results of 4 experimental studies targeting the recovery of energy and carbon from OPEFB and water, energy and oil through direct membrane filtration and a thermophilic AnMBR are presented. Then the results will be extrapolated and integrated, based on which a conceptual typical-sized palmoil-based photobiorefinery will be proposed.

Concentrated solar power-driven gasification of OPEFB, sourced from Malaysia was experimentally tested at the PROMES-CNRS facilities using steam and CO_2 as a gasying agent. Following a DoE approach, it was demonstrated that the reaction could be steered towards H_2- or CO-rich syngas, at cold gas efficiencies of > 100%, owing to the allothermal thermolysis of the OPEFB's moisture, while fine biochar particles could be recovered from the gas stream.

Using both lab-scale PVDF and α-Al_2O_3 UF membranes it was demonstrated that the oily fraction from synthetic Palm Oil Mill effluent could be separated from the permeate, with COD rejection reaching >99.5 % at operational fluxes of 40 and 60 LMH, respectively.

Feeding synthetic POME to a lab-scale thermophilic AnMBR at organic loading rates (2.5 – 4.5 g COD/L· day) demonstrated stable treatment performance, despite LCFA posing a microbial toxicity risk, with > 98 % COD removal, 0.1 g COD/L in the permeate and a volumetric gas production of 11.9 NL/day (48 – 55 % CH_4).

Extrapolating to the typical mill scale, the conceptual integrated photobiorefinery design combining these technologies has a calculated POME and OPEFB recovery potential of 58,940 m^3/yr of reusable water, 35.5 million Nm^3/yr of syngas, 230 ton/yr of biochar, 1 million Nm^3/yr of biomethane, all output representing a >44000 ton CO_2-eq emission reduction potential.

Keywords: Photobiorefinery, solar gasification, thermophilic AnMBR, UF membranes, palm oil.

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SCALING UP A BIOELECTROCHEMICAL BIOFACTORY CONCEPT FROM BREWERY WASTEWATER USING PURPLE PHOTOTROPHIC BACTERIA

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Biorefineries offer a sustainable production model that supports circular economy and enables nutrient recovery and obtention of value-added products from waste feedstocks. The concept of biorefinery model has shifted toward inclusion of more versatile microorganisms to cope with diversity of available waste substrates. Purple phototrophic bacteria (PPB) are particularly suited for this purpose, as they can treat wastewater while producing value-added products such as Biohydrogen, PHA, biomass, protein, Coenzyme CoQ10, carotenoids, lycopene, 5-ALA, biostimulants and microbial fertilizers while growing on a wide range of carbon sources that include wastewaters and more specifically, food industry related wastewaters. Furthermore, PPB can utilize electrodes as extracellular electron donors [1], enhancing the synthesis of these products and allowing metabolical tuning through the electrodes opening the door to a new set of fine tools to work with. In contrast with conventional electrodes (rods, plates) used in electromicrobiology, electrochemical moving bed reactors have been shown to improve PHB production by supporting electroactivity in planktonic cells [2].

To scale up the photoME-MBR concept to a pilot photoME-MB, we used a Bioreactor Büchi CR51 model with a working volume of 50-L (photoME-MBR)50L operated at 30 º C and stirred at 280 rpm using Teflon mixing blades. Mimicking the lab scale configuration, the upscaled system integrated a three-electrode system in which the working electrode was a bed of vitreous carbon (5% v/v) connected to a of a graphite bar as current collector. Biomass production was measured using a standard curve between optical density measured at 590 nanometers (Shimadzu UV-1800 spectrophotometer) and dry biomass. Organic contaminants were analyzed using chemical oxygen demand (COD), total organic carbon (TOC) and total nitrogen (TN) after filtering the samples (0.22 micrometer filter). COD was measured using a commercial kit and analyzed by a HT200S High temperature thermostat and DR3900 Laboratory VIS Spectrophotometer (HACH). Electrochemical measurements and operations were performed using a potentiostat (NEV-4 v2, Nanoelectra S.L., Madrid, Spain).

In this study, a photo microbial electrochemical moving bed reactor (photoME-MBR) was scaled up from 250 mL to 50 L and eventually to 200L which constitutes the largest example for a bioelectrochemically-assisted PPB case study. The new configuration was operated under cathodic conditions to assess i) biomass, PHB, 5-ALA, and CoQ10, ii) brewery wastewater treatment efficiency, and iii) bioelectrochemical performance. Results demonstrated that synthesis of value-added products at pilot scale was comparable to laboratory-scale productivity 1.2 g·L-1 of dry biomass and ca. 110 mgPHB·gDryBiomass-1, while achieving organic pollutants removal at a rate of ca. 136 gTOC/m3·d. Thus, cathodic polarization significantly

enhanced PHB production by promoting extracellular electron uptake from the conductive bed. Microbial community analysis identified Rhodopseudomonas sp. and Bradyrhizobium sp. as dominant genera.

Overall, this study demonstrates the feasibility of scaling up microbial electrochemical moving bed reactors for biorefinery applications. This 200L reactor is compatible and assemblable with a train of treatment which includes a METland® where contaminated water passes through a biofilter material and, after a few minutes of contact, the pollutant load is eliminated by the direct action of the microorganisms. This train of treatment is located currently in IMDEA Water outdoors, serving not only as a single and independent reactor but one step closer to a complete circular and zero wastewater treatment system.

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OPTIMISING SPRAY DRYING TO MAXIMISE ANTIOXIDANT RETENTION IN PURPLE PHOTOTROPHIC BACTERIAL BIOMASS

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Purple phototrophic bacteria (PPB) are a promising microbaial platform for food and feed applications, combining high-protein biomass together with valuable antioxidants, such as carotenoids (CAR) and coenzyme Q10 (CoQ10). These photo-, heat- and oxygen-sensitive compounds enhance nutritional value and shelf-life stability, yet their preservation during downstream processing remains a key challenge. Drying represents the a technical and economic bottleneck, accounting for up to 70% of total downstream processing costs and 95% of utility expenditure. Spray drying offers high throughput and lower costs than freeze or oven drying, yet its impact on antioxidant retention in PPB biomass remains largely unexplored.

Spray drying of PPB biomass was systematically optimized. Three process parameters were investigated: inlet temperature (T_{in}: 90–150°C), feed rate (FR: 20–50 mL·min^{-1}), and dry matter content (DM: 5–25 g·L^{-1}). Spray-dried biomass was benchmarked against freeze-dried control. Car, bacteriochlorophylls (B-Chl), CoQ10, and total antioxidant capacity (TAC) were selected as key response variables to assess antioxidant retention

Feed rate was the main constraint. At FR > 35 mL·min^{-1}, air saturation reduced drying efficiency and increased heat and oxygen exposure, sharply lowering antioxidant retention. Within the viable range (FR ≤ 35 mL·min^{-1}), Tin and DM jointly defined compound-specific optima. Car were best retained at high Tin (95–102°C) with modarate-to-high DM (10–26 g·L^{-1}), whereas B-Chl peaked at lower T_{in} (55–80°C), consistent with lower thermal stability.

TAC proved the most sensitive process indicator, nearly lost under suboptimal conditions and highest at high DM and mid-to-high Tout (65–75°C). A global optimum of FR = 35 35 mL·min^{-1}, T_{in} = 65–70°C, and DM = 18–26 g·L^{-1} maximized retention across all three antioxidant responses. Under optimal conditions, spray drying retained up to 81% and 93.% of CAR and B-Chl respectively, while TAC reached 25.47 ± 2.06 mg AAE·g^{-1}. Under optimized conditions, spray drying matched freeze drying in antioxidant retention and outperformed oven drying. This is, to our knowledge, the first study linking spray drying parameters to antioxidant retention in PPB biomass, supporting industrial application.

Keywords: purple phototrophic bacteria; spray drying; carotenoids; antioxidant capacity

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Section 5.
ADVANCED CONTROL, MODELLING AND ASSESSMENT OF PHOTOACTIVATED PROCESSES

TOWARDS AN UNDERSTANDING OF THE MECHANISMS GOVERNING THE TRANSFER OF ELECTRONS AND PROTONS IN TYPE II PHOTOSYSTEMS.

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Our aim was to investigate the long-range regulatory mechanisms that control the activity of type II photosynthetic bacterial reaction centres (BRCs) with an iron-quinone complex at the acceptor side. These mechanisms are governed by the flexibility of the BRC core proteins, which contain cofactors that participate in electron transport (ET) and residues that are involved in forming a hydrogen network and tuning the redox potential of these cofactors. Our focus was on the role of non-heme iron (NHFe) in these processes. Using the Nuclear Inelastic Scattering (NIS) method, we demonstrated that the stoichiometry of proton uptake and proton transfer reduction in a mutated BRC close to the Q_B site, where two hydrogen bonds were removed (L212Glu/L213Asp to Ala/Ala), and close to the Q_A site, where its hydrophobic character was additionally modified (+M249Ala to Tyr), was directly related to the modification of the partial phonon density of NHFe states. We found that the high spin state of NHFe is important for optimal transfer of fast collective motions on the acceptor side of type II BRCs and for effective ET between quinones Q_A and Q_B.

We believe that long-range coupling of NHFe to the protein matrix, which is regulated by thermal motions, may be crucial for the optimal functioning of BRCs. Understanding how NHFe in BRCs minimises the promiscuity of electron and proton transfer paths is important for designing artificial reaction centres that can perform the fundamental process of photochemical charge separation with comparable efficiency.

Keywords: Bacterial Reaction Centers, dynamic aspects of electron and proton transfer in type II photosystems, long-range coupling.

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OPTIMIZING BIOPRODUCT FORMATION FROM PURPLE PHOTOTROPHIC BACTERIA AND WASTEWATER USING MACHINE LEARNING

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Wastewater can be converted into valuable bioproducts using purple phototrophic bacteria (PPBs). These bacteria utilize light as an energy source, enabling them to efficiently assimilate organic matter while generating bioproducts. Their phototrophic metabolism enables them to grow effectively and outperform other heterotrophic microorganisms in illuminated conditions. PPBs are known for producing high-value products, including hydrogen, polyhydroxyalkanoates (PHA), 5-aminolevulinic acid (5-ALA), coenzyme Q_{10} (Q_{10}), carotenoids, bacteriochlorophylls, pantothenic acid, and protein biomass. Machine learning (ML), a branch of artificial intelligence, helps computers learn from data and perform related tasks. ML algorithms are useful for monitoring and predicting bioproduct production by PPBs, especially given the extensive existing data and diverse operational conditions. This study assesses the performance of three ML models—Random Forest, XGBoost, and CatBoost—in predicting the production of PHA, 5-ALA, Q_{10}, pigments, and biomass. The models were trained on a dataset from prior studies, with input variables including reaction time, organic matter concentration, ethanol, bicarbonate, levulinic acid, ferric citrate, mineral medium, N, C/N ratio, as well as illumination conditions (continuous or intermittent), operation mode (batch or semicontinuous), and volume exchange percentage. The best-performing ML model (CatBoost) was used to determine optimal conditions for each bioproduct using the Particle Swarm Optimization algorithm. Predicted maximum yields were identified for each bioproduct, along with the key factors influencing them. Under the studied conditions, the predicted maximum yields were: 569 mg polyhydroxybutyrate/L, 45 mg polyhydroxyvalerate/L, 79 µmol 5-aminolevulinic acid/L, 13 mg coenzyme Q10/g dw, 7 mg carotenoids/g dw, 17 mg bacteriochlorophylls/g dw, and 2040 mg biomass/L.

Keywords: Photosynthetic microorganisms, Artificial intelligence, Polyhydroxyalkanoates, 5-Aminolevulinic acid, Coenzyme Q_{10}

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METABOLIC FLUX ANALYSIS OF *RHODOBACTER CAPSULATUS* ON PRETREATED VINASSE FOR ENHANCED BIOHYDROGEN PRODUCTION UNDER VARIABLE C/N CONDITIONS

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Metabolic limitations in photofermentative hydrogen production by *Rhodobacter capsulatus* become particularly pronounced when complex wastewaters are used as substrates. Vinasse originating from the yeast, sugar, ethanol or similar industries represents a promising feedstock due to its high organic carbon content. However, its residual nitrogen and heterogeneous composition often favor biomass growth and carbon storage over H_2 evolution. In such systems, the partitioning of cellular resources is governed by the C/N ratio, where nitrogen-rich conditions suppress hydrogen production and promote anabolic pathways, while nitrogen limitation redirects reducing equivalents toward hydrogen production. Concurrently, excess carbon can be diverted toward competing sinks (*e.g.*, poly-hydroxybutyrate (PHB)), further limiting H_2 yields.

To quantitatively resolve these competing pathways, a metabolic flux analysis (MFA) framework is being developed for *R. capsulatus* under photoheterotrophic conditions using pretreated vinasse as the primary substrate. The reconstructed stoichiometric metabolic network will be formulated as a flux balance analysis problem under pseudo steady-state assumptions ($S \cdot v = 0$), with constraints imposed on carbon uptake, nitrogen availability, and photon flux. The model will be supported and calibrated using experimental data to be obtained from batch photobioreactor studies employing both pretreated vinasse and synthetic wastewater formulated to mimic vinasse composition, enabling evaluation under controlled and realistic conditions.

It is expected that under nitrogen-rich conditions, flux will be predominantly directed toward biomass synthesis with negligible H_2 production, whereas increasing the C/N ratio will lead to redistribution of reducing equivalents towards nitrogenase activity, enhancing H_2 production. However, this shift is also anticipated to be accompanied by competition from PHB synthesis, due to its role as a major electron sink. The novelty of this study lies in the development of an experimentally supported MFA framework directly parameterized using vinasse as the model substrate, henceforth establishing a transferable metabolic modeling backbone for wastewater-based photofermentation. By linking influent composition to intracellular flux distribution and product output, the model is expected to support rational reactor and process design without extensive empirical optimization, providing a pathway toward generalized, model driven energy recovery from wastewater systems.

Keywords: MFA, R. capsulatus, wastewater, vinasse, biohydrogen

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SCENARIO-BASED MODEL PREDICTIVE CONTROL OF PURPLE BACTERIA CULTIVATION: SUBSTRATE CHARACTERIZATION FLUCTUATION, VARIABILITY IN LIGHTING CONDITIONS, AND MODEL PARAMETER UNCERTAINTY

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The cultivation of purple phototrophic bacteria (PPB) has been gaining importance as a promising alternative to microalgae for nutrient and resource recovery; yet, its large-scale commercialization remains a challenge. In parallel with investigating the feasibility and commercial expansion of purple phototrophic bacteria (PPB) in full-scale operation and design, attention should also be given to the state-of-the-art in advanced control of cultivation systems. Since the metabolic growth pathways of PPB are highly versatile and influenced by varying biological and operational parameters, the development of a reliable and robust control strategy is essential to enhance process efficiency and product valorization. Therefore, this work presents a scenario-based model predictive control approach for the cultivation of PPB under biological uncertainties and operational disturbances. This approach considers multiple scenarios representing potential uncertainties and disturbances, accounts for different system states under each scenario, and employs an MPC-based framework that optimizes control actions across all scenarios, enhancing robustness to variations and ensuring process efficiency. In the scenario generation, two main sources of variation are considered: (i) substrate characterization fluctuation, and (ii) variability in lighting conditions. Substrate characterization in full-scale plants is often associated with significant uncertainties, which may propagate to other state variables and influence microbial growth pathways. In addition, lighting conditions, such as light–dark cycles, light distribution, and light intensity, can substantially affect process performance at full scale, as they represent the primary driving disturbance. The PAnM model virtually represents mechanistic understanding for PPB growth, yet its kinetic parameters are subject to uncertainty as well. Consequently, in addition to the two aforementioned operational sources of uncertainty, parameter uncertainty is also incorporated into the controller design procedure. Therefore, by realizing scenarios for these sources of uncertainty and incorporating them into the MPC framework, the robustness of PPB production under varying influent characteristics and lighting conditions, as well as model parameter uncertainty can be ensured. The proposed scenario-based MPC approach is then applied to the PAnM model as a simulation benchmark for assessment, and the simulation results demonstrate efficient set-point tracking and robustness to unforeseen uncertainties and disturbances.

Keywords: Purple phototrophic bacteria; robust production; model predictive control; scenario MPC

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UNDERSTANDING METABOLIC SWITCHING: MODELING *RHODOSPIRILLUM RUBRUM* GROWTH RATES

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The ability of phototrophic purple non-sulfur bacteria (PNSB) to switch between metabolic pathways makes them attractive for agro-industrial residue valorization in photobiorefineries. However, these residues vary in composition and concentration. To unlock a wider range of substrates for PNSB cultivation, it is vital to develop a relational understanding between substrate variability, metabolic switching, and observable growth rates. Axenic cultivation with light as a non-limiting substrate ensures dominance of photoheterotrophic growth and product quality control, but is associated with high electricity and heating costs. As shading is inherent to photobioreactors, the parallel metabolic growth constant (Ms) was introduced in the purple bacterial model (PBM) to account for simultaneous photoheterotrophic and chemoheterotrophic growth. Although Ms enabled model validation with experimental data, mechanistic understanding of metabolic switching remains limited.

This work presents the first steps towards the development of a simple, general, and expandable mechanistic growth model to deepen understanding of metabolic switching. Published experimental data on *Rhodospirillum rubrum* were supported by experiments with acetate, CO and light as substrates, using a 1L stirred batch reactor, with light and temperature control, an adapted growth medium. The model describes bacterial growth under anaerobic dark and light conditions and accounts for switching between multiple metabolic states depending on substrate and CO availability. A clear distinction is made between the lag phase, —during which growth is negligible as cells adapt to new environmental conditions—and substrate inhibition occurring at high substrate concentrations, where growth is temporarily suppressed until concentrations decrease below inhibitory thresholds. Half-saturation constants, substrate inhibition coefficients, and lag-phase-related variables were extracted and extrapolated from published studies.

Experimental results demonstrate the conceptual validity of the model under anaerobic light conditions. Still, uncertainties in parameter estimation, particularly for biomass and product yields need improvement. Under anaerobic dark conditions, experimental observations deviated considerably from expectations. Growth appeared to depend primarily on alternative carbon sources, likely the added yeast extract, rather than on CO or acetate. Overall, the framework, despite needing refinement, offers a basis for unifying biological models, that are vital for effective PPB-based valorization of agro-industrial residues.

Keywords: metabolic switch modeling, purple non-sulfur bacteria (PNSB), carbon monoxide

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Section 6.
PHOTOCATALYTIC PROCESSES AND ARTIFICIAL PHOTOSYNTHESIS

SECOND GENERATION BIOMASS-DERIVED COMPOSITE FILMS FOR CIPROFLOXACIN REMOVAL FROM WASTEWATER

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Among the most concerning emerging pollutants in wastewater, an eminent role is played by antibiotics, whose removal cannot be adequately provided by conventional treatment processes. In the context of sustainable water remediation, novel multifunctional materials can be produced using wood from agroforestry waste, exploiting the adsorptive and photocatalytic behavior of cellulose and lignin, which can be effectively isolated using Reductive Catalytic Fractionation (RCF). In this work, the simple and inexpensive hydrotropic (HTNP) and solvent-antisolvent (SASNP) methods were used to convert RCF lignin into nanoparticles, employing nontoxic reagents. These nanoparticles were subsequently combined in a 1% w/w ratio with RCF cellulose to produce nanoparticle-loaded cellulose thin films. These films were put in contact with 5 mg/L solutions of the antibiotic ciprofloxacin (CIP). Circa 25% of CIP was removed by adsorption in 150 minutes, followed by a 14% photocatalytic degradation upon a 150-minute UV irradiation (0.245 $mW \cdot cm^{-2}$), resulting in a total 39% removal. SASNP-loaded films yielded a significantly faster adsorption ($7.6 \cdot 10^{-2}$ min^{-1}) than both HTNP-loaded and pure cellulose films ($1.5 \cdot 10^{-2}$ and $1.1 \cdot 10^{-2}$ min^{-1}, respectively), paralleling the better adsorption performance of SASNPs with respect to HTNPs in aqueous dispersion. No photocatalytic activity was recorded for pure cellulose films, confirming that RCF cellulose acts only as an adsorbent. Conversely, the photocatalytic activity of HTNP-containing films ($5.5 \cdot 10^{-3}$ min^{-1}) was significantly higher than SASNP-containing films ($9.0 \cdot 10^{-4}$ min^{-1}), again confirming the behavior observed in aqueous dispersion. In conclusion, multifunctional composite thin films for ciprofloxacin removal from wastewater were successfully manufactured from second generation lignocellulosic biomass combining innovative biomass fractionation and lignin valorization methods.

Keywords: cellulose, lignin nanoparticles, wastewater treatment, ciprofloxacin, reductive catalytic fractionation

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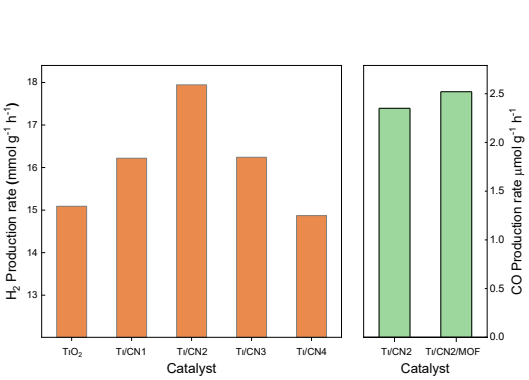
Acknowledgements: This study was carried out in the framework of the project ENCAPSULATE (CUP N. H53D23003890001) (Mission 4 Component 2 Investment 1.1) funded by the European Union Next-GenerationEU – National Recovery and Resilience Plan (NRRP) – Fondo per il Programma Nazionale di Ricerca e Progetti di Rilevante Interesse Nazionale (PRIN).

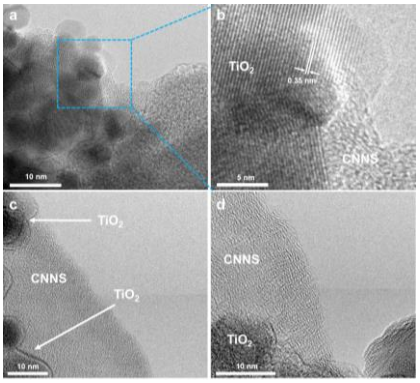
PHOTOCATALYTIC ACTIVITY OF TiO_2/CARBON NITRIDE/MOF TERNARY COMPOSITES FOR CO_2 REDUCTION AND H_2 PRODUCTION

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Heterogeneous photocatalysis offers significant potential for utilizing a free and endless energy source for very converting CO_2 into valuable feedstocks and the production of hydrogen (H_2) as a carbon-free fuel. A wide range of materials has been developed and applied in such reactions. Among them, TiO_2 remains the most extensively studied, primarily due to its low cost and high chemical stability. However, its practical application is limited by inherent drawbacks, including rapid charge-carrier recombination, low surface area, and restricted activity under the solar spectrum. Over the past decade, carbon nitride (CN) and metal–organic frameworks (MOFs) have emerged as promising alternatives for photocatalytic applications especially under visible light irradiation. In this study, in order to develop more efficient photocatalysts, hybrid binary and ternary TiO_2-based composite materials have been developed via the coupling of TiO_2 with CN and/or MOFs structures. All materials were fully characterized using a plethora of characterization techniques including structural, morphological, textural and electronic characterization and tested as photocatalysts for CO_2 reduction and H_2 production under ambient conditions. All composite materials presented higher photocatalytic activity than the parent single phase TiO_2 for both reactions. Results indicate that H_2 production is driven by the ability of the photocatalyst to produce photogenerated electrons while CO_2 is controlled by the ability of the material to adsorb CO_2.





Keywords: Photocatalysis, artificial photosynthesis, CO_2 reduction, hydrogen

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NOVEL APPLICATIONS OF PURPLE BACTERIA

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Research over the past several decades has provided a comprehensive understanding of **bacterial photosynthesis** and the molecular mechanisms governing its **solar light transduction**. This established knowledge base signifies the field's maturity. Consequently, attention is now pivoting towards exploring and implementing applications that leverage bacterial photosynthetic components as **functional soft materials** for roles distinct from their biological function. These efforts are rapidly transitioning from conceptual exploration to tangible applications within material science. This contribution will present a selection of applications relevant to promising new technologies. By addressing both current potential and inherent limitations, this analysis aims to provide a balanced illustration of utilizing the bacterial photosynthetic apparatus and its components in emerging technological domains.

Keywords: Cereibacter sphaeroides, semi-artificial photosynthesis, solar laser

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*Acknowledgements: Project **APACE -** Towards a bio-mimetic sunlight pumped laser based on photosynthetic antenna complexes (Project n. 101161312) funded by Horizon-European Innovation Council is acknowledged.*

Section 7.
MICROBIAL ECOLOGY & PHYSIOLOGY OF PPB AND OTHER PHOTOTROPHS

PHOTOELECTROSYNTHESIS OF CoQ_{10} BY *RHODOPSEUDOMONAS* FIXING CO_2

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Purple phototrophic bacteria (PPB) are promising biocatalysts for sustainable photorefineries due to their ability to fix CO_2 under photoheterotrophic, photoautotrophic and photoelectrotrophic conditions while producing high-value compounds such as coenzyme Q10 (CoQ10). This work evaluates four *Rhodopseudomonas* strains (*Rh. Palustris* TIE-1 and 3 *Rh.* spp. isolated in our laboratory) for biogas upgrading through simultaneous CO_2 conversion and food-grade biomass production. The strains were screened using different electron donors to analyze their ability to fix CO_2, under acetate, butyrate (heterotrophy) and electrochemical-driven (autotrophy) metabolisms. Under acetate, all strains showed superior growth compared to butyrate. Regarding CO_2 fixation in acetate, *Rh. Palustris* TIE-1 exhibited an acclimatization phase and began CO_2 fixation on day 4, while the ambient strains showed CO_2 fixation from day one. In the case of butyrate, all strains fixed CO_2 from day one. Regarding CoQ10 production, acetate as carbon source led to higher yields across all strains, with *Rp.12* exhibiting the highest concentration. In contrast, *Rp.6* showed less difference under both acetate and butyrate, indicating its potential under conditions of excess reducing power. This suggests that *Rp.6* is a promising candidate for the production of value-added compounds under photoelectroautotrophic conditions. To evaluate this, four single-chambers were operated in microbial electrolysis cell (MEC) configuration, applying a constant potential of −0.6 V *vs.* Ag/AgCl at a graphite rod cathode. Total organic carbon (TOC) concentrations were progressively reduced from an initial 8 mM butyrate to identify optimal conditions for CoQ10 photoelectrosynthesis driven by electricity input and CO_2 fixation.

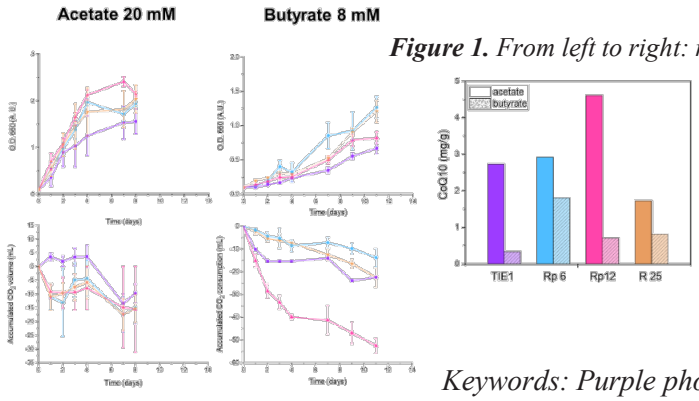


Figure 1. *From left to right: microbial growth and CO_2 fixation using acetate as the carbon source; microbial growth and CO_2 fixation after switching the carbon source to butyrate; and CoQ10 quantification after cultivation with acetate or butyrate as the carbon source.*

Keywords: Purple phototrophic bacteria, biolectrochemistry; CO_2 fixation.

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Acknowledgements: This work has been conducted under the frame of the European project Purple4Life (GA: 101212806), funded by the Circular Bio-based Europe Joint Undertaking (CBE JU) under the European Union's Horizon Europe research and innovation programme.

DECODING ADAPTIVE METABOLISM IN PURPLE NON-SULFUR BACTERIA THROUGH MUTANT LIBRARIES AND FATTY ACID-CENTERED PHENOTYPING

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In purple non-sulfur bacteria (PNSB), fatty acid composition reflects an adaptive metabolic state rather than a fixed cellular trait, changing in response to photoheterotrophic growth conditions and environmental constraints. Resolving how these condition-dependent outputs relate to genetic perturbation is essential to move from empirical cultivation toward function-driven design in photorefinery applications. To address this challenge, a mutant library-based discovery framework is being established in model PNSB, including *Rhodobacter sphaeroides* and *Cereibacter capsulatus*, during a research secondment at Lawrence Berkeley National Laboratory. Strains are evaluated under controlled photoheterotrophic conditions with variable light regimes to induce distinct physiological states. Phenotypic responses are interrogated through fatty acid-centered profiling by GC-MS, providing a robust readout of metabolic remodeling, while complementary high-resolution LC-MS analyses are used for structural refinement and analytical cross-validation. Early observations reveal pronounced strain-dependent divergence in pigmentation dynamics, biomass accumulation, and extracellular matrix formation, consistent with distinct adaptive responses under photoheterotrophic growth. These phenotypic differences support the presence of condition-specific metabolic remodeling and identify fatty acid profiles as a tractable output to connect mutant-derived phenotypes with underlying metabolic functions. By integrating mutant library screening with targeted metabolic readouts, this framework enables the identification of candidate genetic determinants associated with adaptive responses and highlights measurable entry points for metabolic control. This work establishes a scalable genotype-to-phenotype strategy to interrogate adaptive metabolism in PNSB and to identify functional traits relevant to photorefinery optimization. More broadly, it supports the rational engineering of microbial systems toward the production of value-added and potentially bioactive lipid compounds.

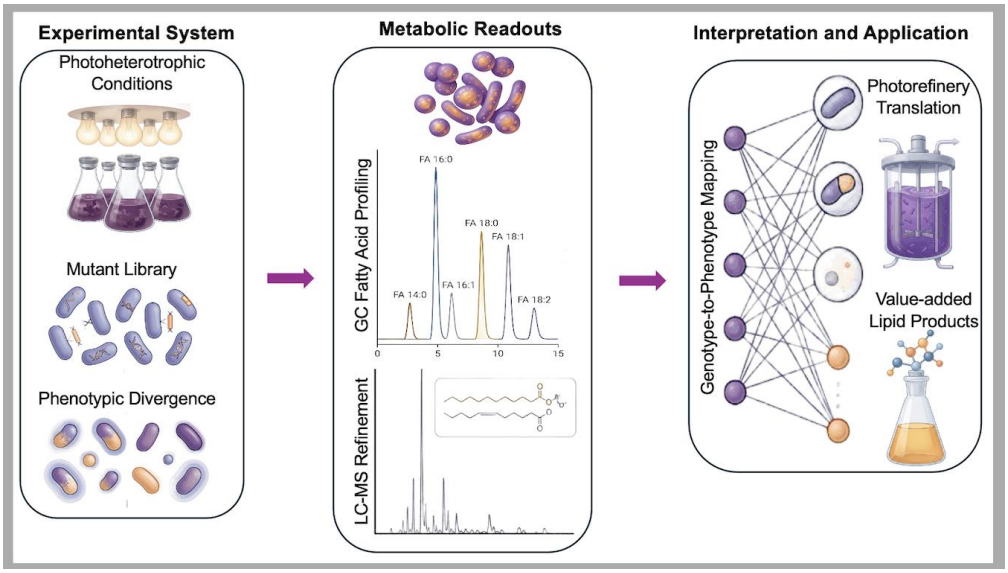


Figure 1. Workflow connecting mutant library screening with fatty acid-centered phenotyping and photorefinery-oriented interpretation in purple non-sulfur bacteria.

Keywords: PNSB, functional genomics, fatty acids, metabolic plasticity, photorefinery

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VALORIZATION OF CHEESE WHEY USING PURPLE PHOTOTROPHIC BACTERIA: APPLICATIONS AND STAKEHOLDER PERSPECTIVES

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Cheese whey is one of the largest by-products of the dairy industry, posing significant environmental challenges due to its high organic load. Nevertheless, its rich composition of lactose, proteins, and minerals makes it a valuable feedstock for biotechnological applications. Purple phototrophic bacteria (PPB) have emerged as a promising microbial platform for whey valorization, capable of converting organic-rich waste streams into biomass and high-value compounds under anaerobic, light-driven conditions. This review addresses PPB-based strategies for cheese whey utilization, including reactor configurations, process optimization, and the technological and stakeholder benefits. The dairy industry produces millions of liters of whey annually. PPB can transform these substrates into protein-rich biomass (single-cell protein), natural pigments, antioxidants, and bioactive lipids, providing opportunities for sustainable food, feed, and bioproduct production. However, several challenges limit the efficiency of whey bioconversion. Most PPB strains cannot directly metabolize lactose, requiring enzymatic hydrolysis or co-culture with lactic acid bacteria. Whey turbidity reduces light penetration, restricting phototrophic activity, while high protein content affects rheology and mass transfer. Pre-treatment strategies such as lactose hydrolysis, acidogenic fermentation, and membrane filtration improve substrate accessibility, and co-culture systems enhance substrate utilization, biomass yield, and metabolic diversity. PPB cultivation has been explored in tubular, flat-panel, and biofilm-based photobioreactors, where light distribution, gas-liquid mass transfer, and biomass retention are critical for scale-up. LED-based illumination, light-dark cycles, and hybrid reactor designs increase phototrophic efficiency. Dynamic modeling and data-driven control strategies support process monitoring, optimization, and automation. PPB-derived biomass exhibits high protein content and a balanced essential amino acid profile, making it suitable for animal feed, aquaculture, and functional food production. Natural pigments, antioxidants, and antimicrobial metabolites expand the range of high-value products. Integration into circular bioeconomy frameworks enhances environmental performance while generating economic opportunities. Future perspectives include the development of lactose-metabolizing PPB strains, optimization of hybrid microbial consortia, and AI-driven process control. Overall, PPB-based whey valorization represents a versatile and sustainable platform, converting dairy waste into functional biomaterials, bioplastics, bioenergy, and nutraceuticals, contributing to circular bioeconomy objectives.

Key words: Whey valorization, Purple phototrophic bacteria, Single cell proteins

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A BIOENERGETIC COMPARISON OF PHOTOTROPHS

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A generalized method for describing microbial metabolism has been well-described for chemotrophs, but is missing for phototrophs. In this study, a thermodynamics approach to determine the theoretical yield of phototrophs in a black-box approach on the basis of the Heijnen dissipation method is proposed and used to compare the bioenergetic efficiencies of PNSB and microalgae.

The complexity of microbial metabolism can be simplified as the sum of **catabolism**, the energy-conserving processes, and **anabolism**, the energy-consuming maintenance and biomass production. In phototrophs, catabolism is the energy conserved through photosynthesis derived from specific wavelengths that initiate charge separation in the photosystem reaction centers and the architecture of the electron transport chain. The energy conserved during photosynthesis is also quantified to represent catabolism. The energy conserving component of the electron transport chain differs between PNSB and microalgae, which use cyclic and linear photophosphorylation, respectively. While the entirety of photon energy that excites an electron can be conserved in PNSB (P870, E = +0.5 V to P870*, E = -0.92 V), the energy-conserving component in microalgae is limited to P680* (E = -0.72 V) and P700 (E = +0.5 V) per photon. Thus, the energy available from 1 mol of photons is -137 kJ $mol_{photons}^{-1}$ in PNSB and -98.4 kJ $mol_{photons}^{-1}$ in microalgae.

Stoichiometric relationships are used to define the anabolic requirements, using various carbon and nitrogen sources to form 1 C-mol of biomass. In our analysis, anabolism is further expanded to include the Gibbs free energy dissipated during microbial growth. The anabolic energy requirements are related to the energy conserved in photosynthesis to derive the metabolic equation through $\lambda_{cat} = \frac{\Delta G_{An} + \Delta G_{Dis}}{-\Delta G_{Cat}}$ using the equation below:

$$Met = \lambda_{cat} \cdot \left[\left(-Y_{Cs/X}^{An*} \cdot C_s - Y_{Ns/X}^{An*} \cdot N_s + C_1H_{1.8}O_{0.5}N_{0.2} \pm Y_{H_2O/X}^{An*} \cdot H_2O \pm Y_{H^+/X}^{An*} \cdot H^+ \pm Y_{e^-/X}^{An*} \cdot e^-\right) + \frac{Y_{e/X}^{An*}}{Y_{e/eD}^{D}} \cdot D\right]$$

Based on the calculated theoretical yields of PNSB and microalgae in their preferred metabolisms, PNSB can produce about 4 times more biomass per mol of photons than microalgae. Although the NIR photons absorbed by PNSB contain less energy than microalgae, they support more biomass yield when photon absorption and charge separation are 100% efficient. An outlook of this study calls for a more accurate quantification of light, beyond photonic energy calculations, to accurately predict biomass yield and determine realistic photon-to-biomass conversion efficiencies.

Keywords: phototrophs, metabolism, thermodynamics, catabolism, anabolism, yield

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HOW PNSB FATTEN UP?

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Purple nonsulfur bacteria (PNSB) are increasingly recognized for their hyperversatile metabolism using light to assimilate organic carbon, nitrogen, and phosphorus found in wastewater. During their preferred metabolism of photoorganoheterophy, electrons are directed towards biomass growth under balanced conditions. However, redox imbalances redirect excess electrons to alternative electron sinks, including the Calvin-Benson-Bassham (CBB) for carbon fixation, the production of polyhydroxyalkanoates (PHAs), H_2, and, more recently, lipids. Lipids, particularly triacylglycerols (TAGs) and wax esters (WEs), have gained considerable attention for their potential use as biofuels or additives to feed or food formulations. Although previously the actinomycetes group were the only described oleaginous prokaryotes, recent studies have demonstrated that PNSB are also capable of accumulating lipids to concentrations between 25 and 35% of dry weight biomass, under high carbon to nitrogen ratios.

This study investigated lipid storage in PNSB under conditions of redox imbalance. Two parallel bioreactors with >700 nm filtered light from halogen lamps were used to selectively enrich for PNSB under anaerobic conditions, at 30°C and pH 7.0 ± 0.1. Acetate ($\gamma_{Cs} = 4$) and butyrate ($\gamma_{Cs} = 5$) served as carbon sources, ammonium as the nitrogen source. Enriched cultures were operated in chemostats under alternating light/dark cycles: (i) 16 h light/8 h dark and (ii) 8 h light/16 h dark, inducing redox stress through carbon accumulation in the dark phases.

We observed that diel cycles impacted the electron distribution in the enrichment. During dark phases, carbon accumulated as PNSB lacked an energy source to assimilate the carbon source. During the light phase, the accumulated carbon was rapidly consumed for biomass production until nitrogen became limiting. Concurrently, reduced substrates generated an excess of electrons, necessitating electron sinks to maintain redox balance with H_2 serving as the primary product.

Under nitrogen-limiting conditions, the total lipid content adjusted for biomass dry weight (*g*) appeared to increase over time (338% and 152%) coinciding H_2 production peaks. Although total lipid content showed a general upward trend suggesting lipid accumulation, the content varied between 5-20% of the cell dry weight with a peak of 25% during the long light cycles. This follows as diel cycles induce transient NADH surpluses, promoting storage compound production. These findings suggest that lipid storage is not the primary response to energy imbalance under these conditions, but contributes to PNSB's metabolic flexibility.

Keywords: PNSB, redox, lipids, resource recovery

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ENVIRONMENTAL STRESS AS A TOOL TO MODULATE CAROTENOID BIOSYNTHESIS AND STRESS RESPONSES IN *RHODOBACTER CAPSULATUS*

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Purple phototrophic bacteria (PPB), such as *Rhodobacter capsulatus*, are promising platforms for circular bioprocessing due to their ability to convert waste into microbial protein and carotenoids. This study evaluates how oxygen availability and UV-C stress affect carotenoid biosynthesis via redox-mediated responses. Pigments were extracted and analyzed using UV–Vis spectrophotometry and HPLC. UV-C exposure decreased cell viability but enhanced carotenoid accumulation by 10–20%. It also caused a temporary growth delay that decreased over successive cycles, suggesting adaptation responses. Oxygen further modulates carotenoid composition through the oxidation of spheroidene to spheroidenone, consistent with previously described CrtA-mediated pathways.

These results demonstrate that controlled environmental stress can enhance carotenoid production in PPB systems. Moreover, the observed adaptive response underscores the potential to optimize cultivation strategies through repeated stress exposure. This finding supports the integration of PPB into scalable bioprocesses for sustainable production of high-value compounds.

Keywords: Rhodobacter capsulatus; Carotenoid production; Oxidative stress; Pigment regulation.

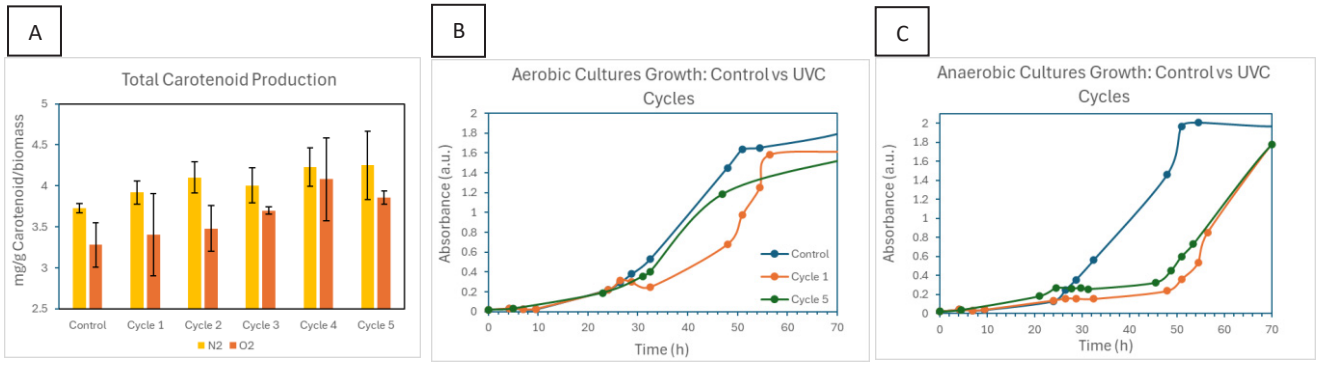


Fig 1. (A) Total carotenoid production (mg carotenoids/g biomass) per cycle under UV-C irradiation compared to non-irradiated controls (N_2 and O_2). (B) Growth curves (absorbance, a.u.) of aerobic cultures for selected cycles vs control. (C) Growth curves (absorbance, a.u.) of anaerobic cultures for selected cycles vs control.

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SHIFTS IN VOLATILE FATTY ACID CO-ASSIMILATION PATTERNS IN *RHODOSPIRILLUM RUBRUM*: REDOX CONSTRAINTS AND SUBSTRATE INTERACTIONS

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Photorefineries aim to valorize diverse organic effluents, including anaerobic fermentation streams, into value-added products (e.g., biomass, high-value metabolites, bioplastics and biohydrogen). Purple phototrophic bacteria (PPB) have emerged as promising platforms for such conversions; however, the mechanisms governing the co-assimilation of VFA mixtures remain poorly understood, as most metabolic studies have focused on single substrates. Under photoheterotrophic conditions, PPB carbon assimilation is tightly linked to redox balance, requiring electron sinks such as CO_2 fixation or H_2 production. VFA assimilation patterns were investigated in *Rhodospirillum rubrum* S1H cultivated on propionate-butyrate mixtures at two total carbon loads (124 or 248 mMnEqC), with electron flow set by bicarbonate ions ($NaHCO_3$) and nitrogen source. Growth, substrate consumption and H_2 production were monitored to assess carbon flux and redox management. VFA co-assimilation followed distinct regimes depending on electron dissipation capacity. Conditions supporting efficient electron sinks (e.g., high $NaHCO_3$ or diazotrophy) promoted sequential assimilation, with propionate consumed prior to butyrate and complete carbon consumption without lag phases (Figure 1 A-D). In contrast, constrained conditions (e.g., low $NaHCO_3$ with high ammonium) led to simultaneous uptake with incomplete carbon consumption, requiring $NaHCO_3$ addition (Figure 1 E-F). This shift highlights a strong link between redox balance and substrate co-assimilation. Within sequential regimes, butyrate assimilation was delayed above a threshold propionate concentration, suggesting competitive interactions. To investigate these dynamics, propionate pulses were applied into cultures actively consuming butyrate to characterize substrate interactions. These patterns likely arise from overlapping pathways, where propionate and butyrate degradation converge toward shared intermediates, leading to enzymatic competition under redox stress. Supporting this, previous observations have shown that the presence of an active glyoxylate shunt (icl^+) may promote simultaneous assimilation. Ongoing work combining kinetics, proteomics and an engineered icl^+ *R. rubrum* strain, alongside comparisons with naturally icl^+ PPB such as *Rhodopseudomonas palustris*, aims to resolve the underlying metabolic control. This work provides new insights into VFA co-assimilation and supports carbon conversion optimization in PPB-based photorefineries.

Purple phototrophic bacteria; volatile fatty acids; co-assimilation; biohydrogen production; resource recovery

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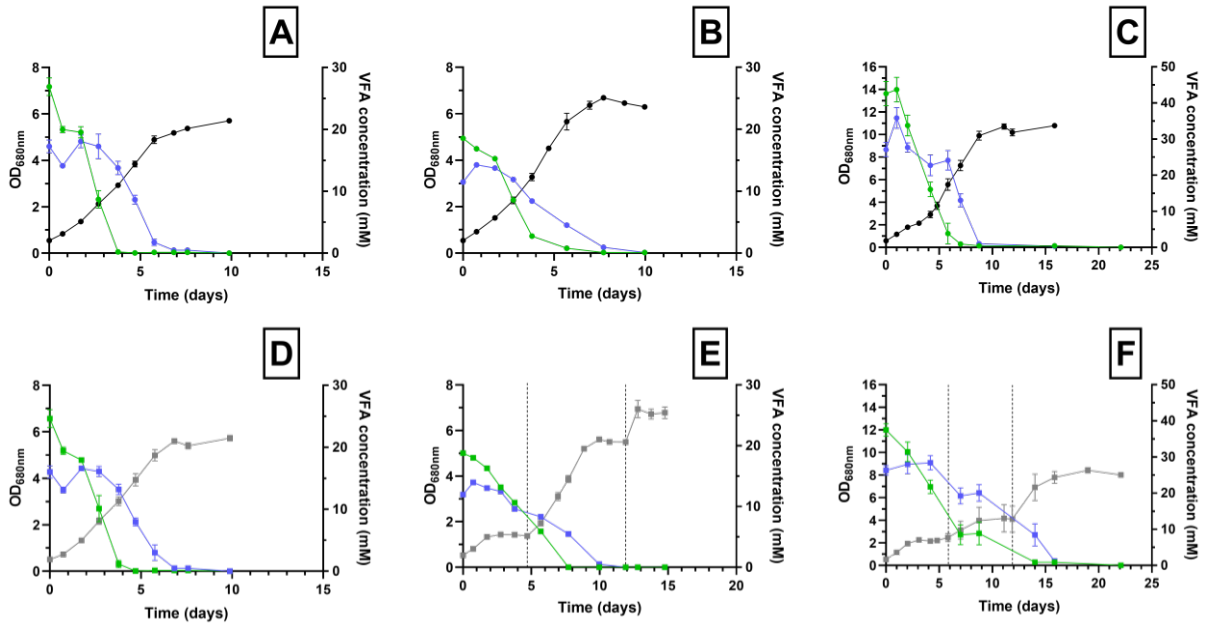


Figure 1. Growth and VFA consumption in *Rhodospirillum rubrum* S1H cultivated on propionate (●) and butyrate (●) mixtures under varying bicarbonate availability (50 vs. 3 mM) and nitrogen sources (N_2 vs. NH_4Cl). Groups: A and D, diazotrophic conditions (N_2) with propionate and butyrate at equal concentrations (62 mMnEqC each); B and E, non-diazotrophic conditions (35 mM NH_4Cl) with propionate and butyrate at equal concentrations (62 mMnEqC each); C and F, non-diazotrophic conditions (35 mM NH_4Cl) with propionate and butyrate at equal concentrations (124 mMnEqC each). Dashed lines indicate bicarbonate additions (5-10 mM) during growth. Data represent mean ± SEM (n = 5).

ECOLOGICAL POTENTIAL OF *RHODOPSEUDOMONAS PALUSTRIS* AS A PURPLE PHOTOTROPHIC DIAZOTROPH FOR SOIL FREE-LIVING NITROGEN FIXATION AND REDUCED-NITROGEN LETTUCE PRODUCTION

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Purple phototrophic bacteria possess versatile photosynthetic metabolism, carbon utilization pathways, and nitrogen transformation capabilities, making them important functional microorganisms for sustainable agriculture. However, their nitrogen-fixing contribution and agronomic relevance in terrestrial soil ecosystems remain insufficiently quantified. *R. palustris*, a representative purple non-sulfur bacterium, has strong potential for free-living nitrogen fixation and plant growth promotion. This study evaluated *R. palustris* as a soil-active PPB diazotroph by integrating $^{15}N_2$ stable isotope tracing, contrasting soil microcosms, and lateritic soil lettuce pot trials.

In sterilized soils, *R. palustris* accounted for more than 99.8% of nifH transcripts, directly demonstrating its nitrogen input as an exogenous phototrophic diazotroph. Net nitrogen fixation increased by 7.31 and 23.87 mg $N \cdot kg^{-1}$ in nutrient-poor acidic lateritic soil and fertile near-neutral phaeozem, respectively. In native soils, inoculation significantly enhanced free-living nitrogen fixation, with strong soil type- and dose-dependence. In phaeozem, low-dose inoculation achieved a net nitrogen fixation increase of 147.01 mg $N \cdot kg^{-1}$, representing an 11.96-fold increase over the control. In lateritic soil, high-dose inoculation produced the highest increase of 7.94 mg $N \cdot kg^{-1}$, indicating that constrained soils require effective establishment of active diazotrophic populations. Path analysis further showed that *R. palustris* exerted the strongest positive direct effect on free-living nitrogen fixation ($\beta = 0.786$, $P < 0.001$), with its abundance and nifH transcript activity serving as key drivers.

To verify agronomic relevance, *R. palustris* was further tested in a lateritic soil lettuce system. Under 30% nitrogen reduction, inoculation maintained high lettuce yield, increased soluble protein content, reduced nitrate accumulation in leaves, and improved nitrogen recovery efficiency and agronomic nitrogen efficiency. ^{15}N labeling showed a low-nitrogen activation and high-nitrogen inhibition pattern, with the highest ^{15}N fixation under reduced nitrogen reaching 7.70 times that of the control. These results demonstrate that *R. palustris* is not merely a plant growth-promoting inoculant, but a soil-active PPB diazotroph with quantifiable nitrogen-fixing contribution, soil-dependent performance, and practical value for reduced-nitrogen crop production. This work expands the ecological relevance of purple phototrophic bacteria from aquatic and wastewater systems to terrestrial agroecosystems and supports PPB-based strategies for soil nitrogen cycling and low-input sustainable agriculture.

Keywords: Purple phototrophic bacteria; Rhodopseudomonas palustris; Free-living nitrogen fixation; $^{15}N_2$ stable isotope tracing; Reduced-nitrogen efficiency

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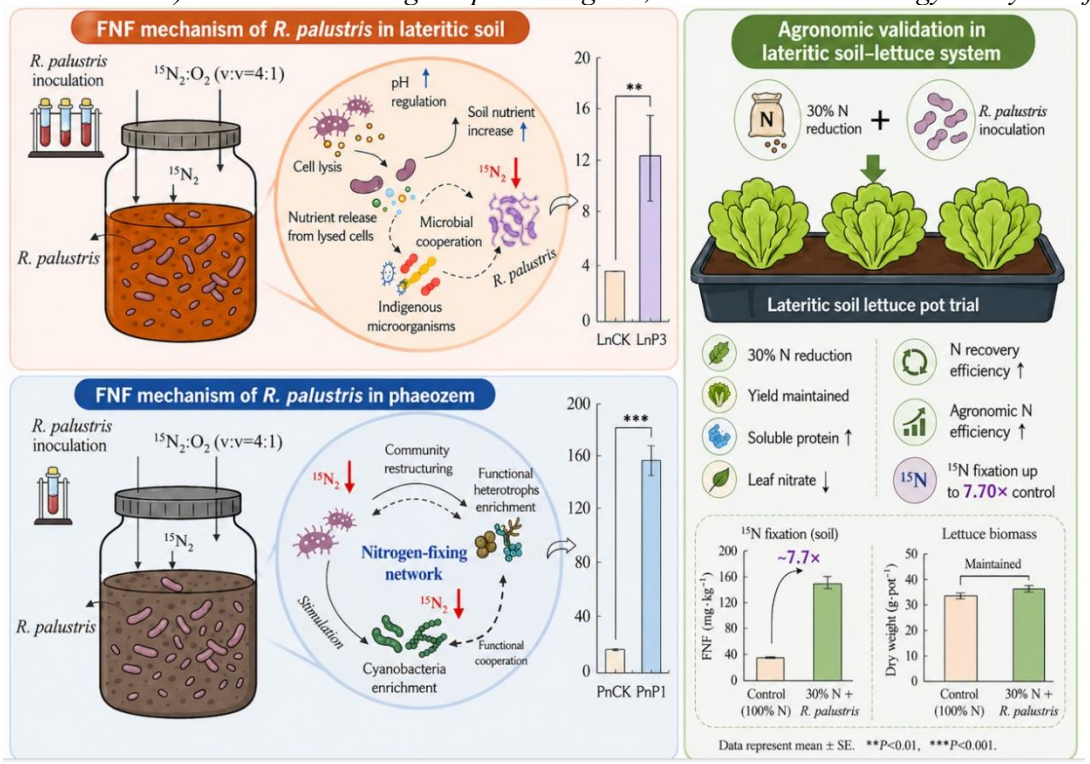


Figure 1. The soil-specific nitrogen fixation and agronomic benefits of *R. palustris*.

Section 8.
TECHNO-ECONOMIC AND LIFE CYCLE ASSESSMENT OF PROCESSESS INVOLVING PHOTO-ACTIVATED PROCESSES IN PHOTOREFINERIES

A MIXED-METHOD CONSUMER ACCEPTANCE AND STAKEHOLDER ENGAGEMENT FRAMEWORK FOR NOVEL PURPLE PHOTOTROPHIC BACTERIA (PPB)-BASED FOOD PRODUCTS

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The transition toward sustainable and circular food systems requires innovative alternative protein and ingredient sources with lower environmental impacts. Previous novel food products, including insect-based foods, have often faced limited acceptance due to social norms, disgust perceptions, taste expectations, and food safety concerns. The Horizon Europe-funded Purple4Life project aims to support sustainable food transitions through the development of food and feed ingredients derived from Purple Phototrophic Bacteria (PPB). PPB are considered promising within the circular bioeconomy due to their metabolic versatility, high carbon conversion efficiency, and ability to valorise diverse by-products into valuable biomass. However, consumer acceptance remains a key factor influencing the successful adoption of PPB-based food products. This study presents the consumer acceptance and stakeholder engagement framework developed within the Purple4Life project. The work examines factors associated with novel food adoption, including food neophobia, perceived safety and naturalness, social norms, cultural differences, trust in production processes, and sensory expectations. A stakeholder micro-mapping approach was used to segment consumers according to demographic, dietary, behavioural, and psychosocial characteristics such as age, dietary preferences, environmental concern, and food neophobia. A mixed-method questionnaire was subsequently developed to assess consumer perceptions and behavioural intentions toward PPB-based food products. The questionnaire combines structured and semi-structured components, including Likert-scale, ranking, and open-ended questions. Survey sections cover demographics, food values, emotional and conceptual associations, situational appropriateness, willingness to consume, sensory expectations, and communication preferences. Responses were transformed into analytical variables and composite indices related to acceptance, perception, and sensory expectations. Preliminary findings indicate moderate-to-positive openness toward PPB-based food products, with willingness to try (3.82/5) scoring higher than purchase or replacement intentions (2.27/5). Environmental concern and increased knowledge about production processes appeared to positively influence acceptance, while perceived safety, trust, and naturalness remained important determinants affecting consumer hesitation. These findings highlight the importance of transparent communication and targeted engagement strategies to support the adoption of microbial-based novel foods. Full results will be presented during the conference. This study contributes to emerging research on sustainable food innovation by proposing an integrated framework for stakeholder engagement, consumer acceptance assessment, and mixed-method analysis of novel microbial food products. The findings may support the development of evidence-based communication and market adoption strategies for PPB-derived foods.

Keywords: Purple Phototrophic Bacteria (PPB), consumer acceptance, novel foods, sustainable food systems, Mixed-Method Analysis

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TAILOR-MADE LED ILLUMINATION FOR PURPLE BACTERIA-BASED PHOTOBIOREFINERIES: HYDROGEN PRODUCTION AND CO-PRODUCT POTENTIAL WITH TECHNO-ECONOMIC ASSESSMENT

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Purple non-sulfur bacteria (PNSB) represent a promising platform for photobiorefinery applications, enabling the conversion of organic substrates into hydrogen and value-added products under mild operating conditions. However, the efficiency of photofermentative processes is strongly limited by light utilisation, making the optimisation of illumination strategies a key challenge.

In this work, *Rhodopseudomonas palustris* was cultivated in continuous photobioreactors and subsequently employed for hydrogen production under tailor-made LED illumination, with emission wavelengths specifically selected to match the absorption peaks of bacteriochlorophylls. This approach improves light utilisation by reducing spectral mismatch between the light source and the bacterial absorption spectrum.

Experimental observations highlighted the influence of spectrum-optimised light on hydrogen production and suggest its potential for integration within multiproduct systems. The results emphasise the role of illumination in improving light conversion efficiency (LCE), which directly affects process productivity and energy performance.

A techno-economic analysis based on experimental data was performed to evaluate process feasibility. Hydrogen production as a stand-alone product resulted in a levelized cost of hydrogen (LCOH) in the range of 120–450 € kg^{-1}, indicating limited economic viability. The inclusion of co-products such as PHB could improve the overall process performance, supporting the transition from a single-product system to a photobiorefinery concept.

Overall, these results suggest that combining spectrum-tailored illumination with product diversification could represent a promising strategy to enhance the sustainability of photofermentative processes and to enable their integration into circular bio-based systems.

Keywords: Photofermentation, Purple non-sulfur bacteria, Light conversion efficiency (LCE), Tailor-made LED illumination.

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REDEFINING MOLASSES AS A FEEDSTOCK FOR *RHODOSPIRILLUM RUBRUM* PROTEIN PRODUCTION: A TECHNO-ECONOMIC AND LIFE CYCLE PERSPECTIVE

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The transition toward a circular bioeconomy necessitates the strategic upcycling of industrial side-streams into high-value products to meet the protein demands of a global population projected to reach 10 billion by 2050. While sugar beet molasses is traditionally relegated to low-value applications or energy recovery through photofermentation for hydrogen production, this study establishes a comprehensive framework for its "nutritional upcycling" into high-quality microbial protein using a glucose-acclimated strain of the purple non-sulphur bacterium *Rhodospirillum rubrum*. Our findings demonstrate that optimized cultivation can yield a biomass with protein content exceeding 55% and a superior essential amino acid profile (EAAI > 1), positioning it as a competitive alternative to conventional animal proteins. To evaluate the industrial viability of this transition, a dual-modelling approach was employed, integrating Techno-Economic Analysis (TEA) and Life Cycle Assessment (LCA). The TEA identifies illumination energy and labour as the primary economic drivers, accounting for approximately 90% of the cost variance. Specifically, optimizing biomass concentration from bag photobioreactor levels to those achieved in laboratory settings can reduce medium preparation costs by 73% (dropping to 2.02 €/kg), while the implementation of advanced LED technology could lower illumination costs to approximately 11.57 €/kg (Figure 1). Complementary LCA results reveal that the carbon footprint of this microbial protein is already six times lower than that of conventional bovine protein. Under optimized scenarios, including high productivity and 10-fold improvements in energy efficiency, the environmental footprint can be divided by more than 20 for almost all indicators compared to initial experimental setups (Figure 2) Furthermore, the analysis highlights that cascading molasses into protein-rich biomass offers superior systemic environmental benefits compared to its use for hydrogen production. Ultimately, this research provides a scalable roadmap for integrating photosynthetic bacteria into sustainable food systems, demonstrating that aligning industrial residue management with global protein security is both economically promising and environmentally imperative.

Keywords :Rhodospirillum rubrum; Single-cell protein; Circular bioeconomy; Techno-economic analysis; Life cycle assessment

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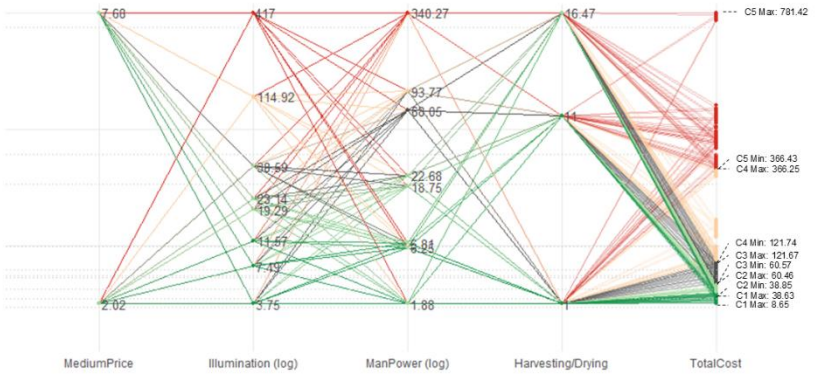


Figure 1 : Parallel coordinates plot showing the relationship between MediumPrice, Illumination (log), ManPower (log), Harvesting, and TotalCost across scenarios. Each line represents one scenario, and colors indicate cluster groups (C1–C5). The ranges of TotalCost for each cluster are indicated on the right axis.

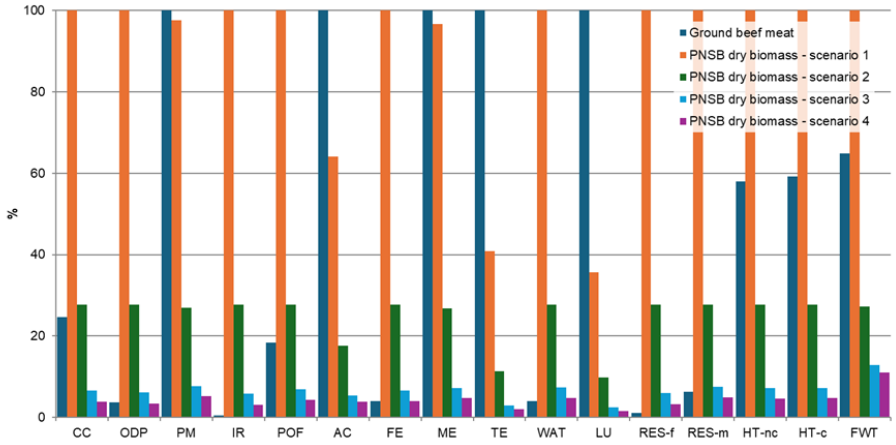


Figure 2: Life cycle assessment (LCA) comparison of beef protein (blue) and purple bacteria–based protein under different scenarios: low productivity with halogen lighting (orange), high productivity with halogen lighting (yellow), and high productivity with optimized LED systems (light green: 5× optimisation; dark green: 10× optimisation) considering a functional unit of 1kg of protein. CC: climate change; ODP: Ozone Depletion Potential; PM: Particulate Matter; IR: Ionising radiation; POF: Photochemical Ozone Formation; AC: Acidification; FE: Freshwater Eutrophication; ME: Marine Eutrophication TE: Terrestrial Eutrophication; WAT: Water Use; LU: Land Use; RES-f: Resource depletion – fossil; RES-m: Resource depletion metal ; HT-nc: Human toxicity non-cancer; HT-c: Human toxicity cancer; FWT: Freshwater Ecotoxicity.

Section 9.
PHOTOREACTOR DESING: CHALLENGES AND FUTURE DIRECTIONS

3D-PRINTED BIORECEPTIVE CLAY PANELS AS OPEN BIOREACTORS INFORMED BY ALGAL BIOFILM COLONIZATION

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Microalgal biofilms naturally colonise porous mineral substrates, where substrate mineralogy, moisture and light create stable habitats. Clay mineral surfaces are particularly promising microniches due to their nutrient-richness and moisture retention. Yet conventional building-integrated algal systems rely on closed photobioreactors with complex energy-intensive infrastructure, which limits architectural integration and scalability. This motivates the need for low-energy, passively bioreceptive façade elements that leverage natural colonisation mechanisms.

Here, we present an open bioreceptive façade element made of clay – a material that can be designed using advanced computational systems to generate attractive appearances and complex topologies. Such computational design not only optimizes bioreceptive performance (e.g. surface roughness, moisture distribution) but also enhances building aesthetics. We develop 3D printed bioreceptive clay panels acting as open photobioreactors that mimic natural algal colonisation on mineral substrates, targeting façade-scale applications such as air pollution monitoring and bioremediation. Using *Chlorella vulgaris* as a model organism with a demonstrated capacity for CO2 capture and air-pollutant remediation, we evaluate clay formulations under façade-relevant environmental conditions. 3D printing creates layered topographies that increase surface roughness and improve moisture distribution, thereby promoting algal attachment. The design incorporates vertical substrates with drip irrigation and a lower collection zone where nutrient medium accumulates transiently, supporting both attached biofilms on porous clay and suspended planktonic cultures. A low-energy recirculation loop permits periodic biomass harvesting while reseeding the irrigation line to sustain mixed-mode colonisation.

Colonisation dynamics and biomass yield are quantified via growth measurements, surface coverage analysis and microscopy. Initial results show that porous, water-retentive clays support persistent biofilm formation, and the collection zone enables controlled planktonic biomass harvesting. This system offers a low-energy, mineral-based alternative to glazed photobioreactor façades, integrating microalgal photorefineries directly into building envelopes.

Keywords: bioreceptive ceramics, open photobioreactor, building-integrated algae, microalgae biofilm, digital fabrication

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PHOTOREFINERIES TO INCREASE THE SUSTAINABILITY OF THE AGRI-FOOD SECTOR. CHALLENGES AND OPPORTUNITIES.

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The agri-food sector is highly relevant, especially in regions such as Catalonia (Spain), where it contributes to 19% of their total GDP. This implies that livestock waste and by-products are generated in large quantities, with high potential for recovery. The anaerobic digestion (AD) of these by-products is a well-known process allowing the production of a renewable biofuel (biogas or biomethane) in quantities around 250-300 L CH_4/kgVS in the case of cow manure. A critical issue in AD is the management of digestates. Uncontrolled digestate management has generated significant ecological impacts such as eutrophication and nitrate contamination of groundwater bodies. Digestates are normally handled by energy-intensive processes with limited capacity to recover valuable compounds. As alternative, the use of photosynthetic organisms in "Photorefinery" schemes is a more sustainable option that fit within the circular economy framework. Digestates can act as a nutrient source for the growth of photosynthetic cultures, i.e., purple phototrophic bacteria (PPB) and/or green microalgae; allowing the recovery of carbon, nitrogen and phosphorus. Photosynthetic biomass is then used to generate bio-products such as agricultural biostimulants, biofertilisers and alternative protein for animal feeding.

Green microalgae can produce biomass more efficiently than terrestrial plants, also serving as CO_2 sink. Extrapolating our pilot-scale results to industrial scale, if a daily flow of 100 $m^3 \cdot d^{-1}$ of livestock waste would enter an industrial digester of 6000 m^3, mass balances would indicate productions of 914-1059 $Nm^3CH_4 \cdot d^{-1}$ of biomethane (as biogas), and 120 $t \cdot d^{-1}$ of microalgal biomass to be potentially used as plant biostimulant. Biostimulants have an economic value around 10 times higher than biofertilisers and higher market uptake potential. Germination tests of wheat seeds using biostimulants from microalgal biomass (grown in digestate) showed 115% of germination index (compared to control). However, the digestate used to grow microalgae was highly diluted (<5%), implying limited capacity of microalgal systems to treat digestates. As an alternative, PPB can grow at NH_4 concentrations >1500 mgN-NH_4/L. Our previous studies showed PPB growing in bench-scale flasks containing 10-50% of digestate. PPB's growth rates accounted for 0.2-1.9 d^{-1}, which were considerably higher than those of microalgae: 0.13-0.19 d^{-1}. However, their N-removal capacity is lower than microalgae, due to their lower nitrogen content. In fact, typical C:N:P of PPB is 100:7:1 (106:16:1 for microalgae). This caused that the protein content of PPB biomass (30-38%) is lower than that of microalgae (40-45%). Further research is needed to understand which conditions are more favourable for each photosynthetic organism, with the goal to optimise nutrient recovery from digestates and biomass production for obtaining high-quality bio-products. But to further implement these technologies, it is essential to evaluate the regulatory frameworks and market uptake of these bio-products, which is a current challenge.

Keywords: agro-industrial waste, biostimulant, digestate, microalgae, purple phototrophic bacteria.

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DUAL LAND USE FOR FOOD AND ENERGY: PHOTOVOLTAIC INTEGRATED BIOPROCESSES FOR SINGLE-CELL PROTEIN PRODUCTION

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Food security is an urgent challenge as the global population grows and demand for protein increases. With conventional production reaching resource limits, the protein transition toward sustainable alternatives such as single-cell protein (SCP) requires scalable and land-efficient production systems. Large-scale phototrophic cultivation for SCP is frequently constrained by land availability and cost, a key bottleneck for industrial scale-up. Paradoxically, vast areas of land are being converted to PV solar farms, where land acquisition appears economically feasible, in part due to energy subsidies [1]. By integrating phototrophic bioprocesses with solar infrastructure, the same land can simultaneously produce renewable electricity and microbial biomass, with low-cost organic waste streams adding further economic merit.

This study investigates the design of a system in which semi-transparent, wavelength-selective PV panels are integrated with photobioreactors for purple non-sulfur bacteria (PNSB) cultivation, alongside a quantitative analysis of land-use and energetic efficiency for PV-driven SCP compared to conventional agriculture. Purple phototrophic bacteria are preferred over microalgae due to their rapid growth and metabolic versatility, which may support more efficient and scalable biomass production [2].

By transmitting specific wavebands while converting the remaining spectrum to electricity, the PV layer creates a spectrally defined light environment inside the reactor. This spectral filtering acts as a continuous selective pressure on an open culture system, favouring PNSB over competing phototrophs without sterile operation, an important attribute for large-scale deployment. Biomass yield (η_bio) is evaluated across organic substrates including volatile fatty acids, sugars, carbohydrates, and amino acids, and together with photovoltaic efficiency (η_pv), defines the overall energetic performance of the system. The study examines PV configurations, including material choices and wafer spacing, and reactor configurations suited to outdoor, open operation, with spectral transmission profiles and culture dominance through light-mediated selection as key parameters.

Overall, this work presents dual land use as a compelling framework for sustainable SCP production, where solar energy infrastructure and microbial cultivation are evaluated to maximise resource efficiency and land productivity.

Keywords: SCP, Photovoltaic, Photobioreactor

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POSTER SESSION

CUSTOM-BUILT LED-ILLUMINATION SETUP FOR PURPLE BACTERIA

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The light-harvesting complexes of purple photosynthetic bacteria have an absorption maximum in the near-infrared region. In the lab, illumination with tungsten light-bulbs used to be very convenient for optimal growth. However, after those bulbs were phased out by European law (due to energy conserving reasons), alternatives have to be found.
Here we describe the development of illumination devices using low-energy LEDs, which were optimized for the purple bacterium *Rhodospirillum rubrum*. Different wavelengths are combined to yield optimal growth conditions. Additionally, pulsed illumination was applied in order to get some information about time-dependent energy transfer processes in the cells.

Keywords: Rhodospirillum rubrum, low-energy LEDs, near-infrared LEDs, pulsed illumination

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A CIRCULAR ECONOMY APPROACH: ASSESSING THE ENVIRONMENTAL PROFILE OF MICROALGAE-BASED FOOD AND FEED PRODUCED FROM INDUSTRIAL WASTEWATERS

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Spain is the second largest beer producer in the European Union (4.0 billion liters) and sixth largest cheese producer (538,210 tons). These industries are largely driven by small and medium-sized enterprises that have difficulties on managing their effluents. Brewery wastewater and cheese whey are nutrient-rich streams that offer an ideal substrate for microalgae cultivation generating a biomass with applicability in different industrial sectors. The objective of this study is to performa Life Cycle Assessment (LCA) to evaluate and compare the environmental impacts of microalgal biomass into value-added products. The LCA methodology was developed using SimaPro software to model and analyze four distinct scenarios. The scenarios analyzed were cheese whey (CW) for biscuits production (B) (scenario 1 - CH-B) and fish feed (F) (scenario 2 - CH-F) and Brewery wastewater (B) for biscuits and fish feed (scenario 3 - BR-B, Scenario 4 - BR-F). These scenarios use data from previous cultivation and harvesting of *Scenedesmus* sp. in brewery wastewater and, *Tetraselmis chuii* grown in digested cheese whey The system boundary includes the cultivation, harvesting, downstream processing, and final product manufacturing stages. In conclusion, this assessment identifies environmental impacts and the benefits of utilizing microalgal biomass, while highlighting the differences between the food and feed scenarios. These outcomes will support small-scale food industries in transitioning towards microalgae-based circular economy.

Keywords: Circular Economy, Life Cycle Assessment, Scenedesmus sp., Tetraselmis chuii, Wastewater Valorization

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EFFECT OF ACCLIMATION ON GROWTH AND NUTRIENT REMOVAL OF NATIVE MICROALGAE POLYCULTURE GROWN IN ANAEROBIC SANITARY WASTEWATER FROM DIFFERENT TREATMENT PLANTS

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Acclimation promotes the adaptation of microalgae strains, but it is scarcely reported for polyculture treatment in wastewater. Hence, this study evaluated the effect of acclimation on two sanitary anaerobic wastewaters from Candeia (CWW) and Tibiriçá (TWW), both located in the central-west region of São Paulo state, Brazil. Four experimental conditions were tested at a laboratory scale: B1 (TWW and native microalgae polyculture inoculum grown in TWW), B2 (CWW and native microalgae polyculture inoculum grown in TWW without acclimation), B3 (CWW and native microalgae inoculum grown in TWW and acclimated in CWW), B4 (CWW and native microalgae polyculture inoculum grown in CWW). Biomass productivity was statistically similar across conditions ($p > 0.05$), ranging from 0.10 to 0.14 g L^{-1} d^{-1}. Although the lag phase (λ) varied significantly between B2 (0.88 d) and B3 (0.77 d) ($p = 0.001$), the maximum specific growth rate was minimally impacted (B2: 0.89 d^{-1}; B3: 0.86 d^{-1}; $p > 0.05$). The similarity in system performance was reinforced by an effect size of < 0.2 (ranging from 0.02 to 0.07) for total phosphorus (TP), total nitrogen (TN), optical density (OD_{680}), and total suspended solids (TSS). Removal efficiencies of ≥ 93% and ≥ 90% were achieved for TP and TN, respectively, across B1 to B4. These similar growth rates demonstrate that sanitary anaerobic wastewaters with comparable physicochemical properties can be successfully treated without acclimation, thereby saving time and resources.

Keywords: Acclimation, Effect size, Native microalgae polyculture, Anaerobic sanitary wastewater, physico chemical properties.

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ANAEROBIC CO-DIGESTION OF WASTEWATER ALGAE FROM THREE WASTEWATER TREATMENT CONFIGURATIONS WITH FAT, OIL AND GREASE

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This study investigates anaerobic co-digestion of fat, oil and grease (FOG) with three wastewater (WW) alga types to support integrated waste and wastewater treatment for energy and resource recovery in urban and regional contexts. Pilot-scale algae treating three different types of wastewaters, anaerobic digestion centrate (centrate-algae), raw wastewater (photogranules), and UASB effluent (UASB-algae) were co-digested with FOG at ratios ranging from 100:0 to 0:100. Methane yields and process kinetics were evaluated using globally fitted first-order models. Significant variation was observed among alga types, with hydrolysis rates of 0.10, 0.24, and 0.37 d-1 and methane yields of 321, 251, and 142 L CH4 kgVS-1 for centrate-algae, photogranules, and UASB-algae, respectively. Methane yields during co-digestion were largely additive, showing no clear synergistic effects and a strong linear relationship with mixture C/N ratio. In contrast, FOG addition substantially enhanced digestion rates; for example, co-digesting centrate-algae with FOG increased the rate from 0.10 to 0.28 d-1, potentially reducing sludge retention time from ~70 days to <15 days. Ammonia inhibition and integration implications for full-scale application are also discussed.

Keywords: Photogranules, Algae co-digestion, Algae wastewater treatment, Fat Oils and Graese (FOG), Bioenergy

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ANODIC POTENTIAL AS A CONTROL PARAMETER IN PPB PHOTO-BIOELECTROCHEMICAL VALORIZATION OF WAO-TREATED REFINERY SLUDGE

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The valorization of refinery oily sludge remains challenging due to its hazardous nature, high organic load and complex hydrocarbon-rich composition. In this work, purple phototrophic bacteria (PPB) were integrated with wet air oxidation (WAO) and photo-bioelectrochemical operation to evaluate whether anodic polarization can steer the conversion of refinery sludge-derived organics towards treatment- or valorization-oriented outputs. WAO was first used to oxidize and solubilize high molecular weight hydrocarbons from refinery oily sludge, generating an aqueous phase suitable for PPB cultivation. This WAO effluent contained high soluble chemical oxygen demand (SCOD ≈11.4 g L^{-1}) and ammonium concentration (NH_4^+ ≈0.84 g L^{-1}) and was diluted to 33% (v/v) to reduce microbial inhibition while preserving substrate availability. The diluted stream was treated in H-type photo-microbial electrolysis cells inoculated with an enriched natural PPB culture. Graphite anodes were poised at +0.2, +0.4, +0.6 and +0.8 V vs Ag/AgCl, using platinized titanium mesh cathodes and infrared illumination. Open circuit potencial reactors (OCP) were operated as non-polarized controls.

Electrode polarization clearly modified the fate of the soluble organic fraction. After four days, SCOD removal increased from 22% in OCP to 35–43% under polarized operation. The +0.8 V condition showed the highest removal at this stage and reached the maximum overall SCOD removal of the assay at the end of the experiment (52%). In addition, final residual SCOD under polarization was 21–32% lower than in OCP, while polarized reactors maintained measurable late-stage activity after the control approached near-zero consumption. Ammonium removal was also enhanced, increasing from 12% in OCP to 27–34% under polarized conditions. However, the potential that maximized organic conversion was not the one that maximized valorization products. Planktonic biomass reached its highest value at +0.4 V (800 ± 100 mg VSS L^{-1}), while it decreased to 660 ± 50 mg VSS L^{-1} at +0.8 V. Apparent biomass yield was 12% higher than OCP at +0.4 V but 32% lower at +0.8 V, indicating a shift from biomass formation to electrode respiration at more oxidizing potentials. Biofilm development and the carotenoid production were highest at +0.2 V.

Overall, anodic potential acted as a key operational lever in PPB photo-bioelectrochemical systems: +0.8 V promoted organic conversion and ammonium removal, whereas +0.2–+0.4 V favoured biomass formation, biofilm development and pigment-oriented valorization.

Keywords: Purple phototrophic bacteria; Wet air oxidation; Photo-bioelectrochemistry; Refinery sludge; Carotenoids

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LAYERED DOUBLE HYDROXIDE-DERIVED MIXED OXIDES FOR PHOTOCATALYTIC DEGRADATION OF ORGANIC POLLUTANTS: LINKING STRUCTURE/TEXTURE TO PERFORMANCE

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The development of efficient photocatalysts for wastewater treatment requires in-depth investigation of their properties that govern photocatalytic performance. In this study, Zn-based layered double hydroxides (LDHs) containing different trivalent metal cations were designed as precursors for the preparation of mixed metal oxides with photocatalytic properties. ZnAl, ZnCr and ZnFe systems were synthesized by the coprecipitation method and thermally treated at 500 °C and 900 °C in order to investigate the influence of metal composition and calcination temperature on their properties and photocatalytic efficiency. The materials were characterized using X-ray diffraction and low-temperature nitrogen adsorption. XRD analysis confirmed the formation LDH-phases and their transformation into mixed metal oxides after calcination or spinel phases. Textural analysis revealed that thermal treatment at 500 °C promoted the formation of well-developed mesoporous structures and increased BET surface area only for ZnAl system. However, for ZnCr and ZnFe systems thermal treatment at 500 °C caused the disappearance of smaller mesopores leading to the decrease of BET surface area. Calcination at 900 °C initiated crystallite growth and low BET surface area in all systems. These structural changes directly affected surface reactivity, charge separation and accessibility of photocatalytic active sites. The photocatalytic efficiency of all synthesized materials was evaluated through the photodegradation of Rhodamine B (RhB), Methylene blue (MB) and Brilliant cresyl blue (BCB). The results demonstrated that photocatalytic efficiency strongly depended on catalyst composition and thermal treatment conditions. It was observed that the highest photodegradation efficiency of ZnAl, ZnCr and ZnFe systems differed for all three model pollutants, demonstrating that the photocatalyst performance is also pollutant-dependent. The highest efficiency toward RhB degradation (~100%) exhibited ZnAl 500, for MB degradation superior performance showed ZnCr 900 (~100%), whereas ZnFe 900 achieved the highest degradation efficiency for BCB (~100%). Kinetic analysis revealed that photodegradation reactions followed pseudo-first-order model. The established relationships among structural and textural properties and photocatalytic performance demonstrate that the rational engineering of LDH-derived mixed oxides through compositional design and controlled thermal treatment enables the development of highly efficient photocatalysts. The obtained findings provide valuable guidelines for the design of multifunctional materials for sustainable wastewater treatment and advanced environmental remediation, contributing to the development of next-generation photocatalytic technologies.

Keywords: Zn-based photocatalysts, photocatalysis, wastewater treatment, organic dyes

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EXERGY-, EXERGOECONOMIC- AND LIFE-CYCLE-BASED EVALUATION OF PHOTO-ACTIVATED PROCESSES IN PHOTOREFINERIES

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Photorefineries utilise solar energy to extract carbon, nutrients, and energy from waste streams by linking solar radiation to photocatalysts or phototrophic microorganisms. Their performance is typically evaluated by energy balances, techno-economic metrics (CAPEX, OPEX, levelized cost), and life cycle assessment (LCA). These metrics, however, neglect the thermodynamic quality of the conversion: a significant portion of the exergy from incident solar radiation is dissipated within the photoreactor due to optical losses, charge-carrier recombination, and reaction irreversibilities, while energy-based efficiencies fail to identify these losses. This study proposes an integrated exergy-exergoeconomic-LCA framework for photo-activated resource recovery systems and demonstrates its application to food-processing waste feedstocks. Exergy analysis quantifies and identifies irreversibilities; the exergy efficiencies reported for photocatalytic hydrogen reactors are small, approximately a few percent, indicating that the photoreactor serves as the principal exergy sink and a key focus for enhancement. Exergoeconomics assigns a monetary value to exergy streams and exergy destruction, highlighting optimal capital allocation. This aligns with techno-economic evidence indicating that the economics of full-scale photocatalytic systems are primarily influenced by the catalyst-replacement interval and electricity tariffs, rather than incremental catalyst activity, and that the levelized cost of photocatalytic hydrogen (4.9-7.8 USD kg^{-1}) is predominantly determined by capital and labour costs. LCA situates these losses within a broader environmental framework: for photo-activated processes, the operational phase (electricity for illumination and pumping) constitutes the majority of the impact and cost; solar photo-Fenton associated with biological treatment exhibits 80–90% lower impacts compared to heterogeneous photocatalysis; and substituting grid power with solar energy or photovoltaics serves as the most significant lever, diminishing the climate-change impact by approximately 93%, albeit transferring the burden to mineral-resource depletion. Artificial illumination constitutes the most significant operational expense (1.9 USD kg^{-1} dry biomass) for phototrophic pathways; thus, sunlight-based external operations are essential for scalable practicality. The three methods could be integrated via a shared exergy-based functional unit (exergy, or cost per unit exergy, of the recovered product) to address the methodological heterogeneity-divergent functional units, system boundaries, and impact assessment methods-that presently constrains cross-study comparison. The proposed framework provides thermodynamically consistent decision assistance for the design and scale-up of photorefineries that valorise food-processing waste sustainably and competitively.

Keywords: Exergy analysis, exergoeconomics, life cycle assessment, photo-activated processes, photorefinery

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PAN-LIFE-CARPET AS AN INTEGRATED BIOREFINERY STRATEGY FOR LAND RESTORATION

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Pan-Life-Carpet (PLC) (Palanački Malešević et al., 2024) represents a strategy designed to address major environmental challenges in drylands, particularly land degradation and associated deterioration of air and water quality. The PLC concept is based on the accelerated biocrust development, in which cyanobacteria act as pioneer biocrust-forming organisms. PLC combines organisms (i.e., cyanobacteria) with polysaccharide and physical supports aimed at mitigating harsh environmental conditions in drylands.

The cyanobacterial inoculum within the PLC concept could be obtained from biomass cultivated in nutrient-rich wastewaters or cyanobacterial blooms from eutrophicated aquatic ecosystems. Cultivation in wastewaters contributes to nutrient removal, while harvested blooming biomass supports eutrophication mitigation. Polysaccharide support enhances moisture availability and adhesion of organisms to soil particles. Obtaining these biopolymers through microbial cultivation in nutrient-rich wastewater-based media additionally contributes to wastewater treatment and waste valorization, with xanthan representing a promising candidate due to its high-water absorption capacity and adhesive properties. Physical support provides initial soil stabilization and facilitates large-scale inoculum application, while biodegradable materials such as jute derived from agro-industrial waste additionally contribute to waste valorization.

Overall, the PLC represents an integrated nature-based strategy with a biorefinery approach aimed at dryland restoration. Utilizing blooming biomass, wastewater-derived cyanobacterial biomass and polysaccharides, as well as biodegradable agro-industrial materials, PLC supports waste valorization, improves water and air quality, and promotes circular bioeconomy principles.

Keywords: land degradation, waste valorization, circular bioeconomy, biocrusts, cyanobacteria

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MATCHING BIOMASS VALORIZATION AND BIOHYDROGEN PRODUCTION THROUGH PURPLE PHOTOTROPHIC BACTERIA

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Given the diminishing availability of fossil fuels and their contribution to climate change, green hydrogen presents a promising alternative for the future, due to its high specific energy content, and the fact that residues from burning this fuel are less harmful for the environment in a very large extent. Unfortunately, nowadays, still 90% of the world's hydrogen is being produced precisely from fossil fuels. Biological hydrogen production or biohydrogen, understood as hydrogen produced chemically, thermochemically, biologically, biochemically, and bio-photolytically from all biomass materials, represents a potentially sustainable alternative, particularly when coupled with the valorization of renewable biomass residues.

Purple phototrophic bacteria (PPB) constitute a metabolically versatile group of facultative anaerobic microorganisms capable of utilizing infrared light as an energy source while producing hydrogen through nitrogenase-mediated pathways. Compared to other phototrophic microorganisms, PPB exhibit high growth rates and reduced sensitivity to oxygen, making them attractive candidates for biohydrogen production systems. Nevertheless, hydrogen generation by PPB is strongly dependent on environmental and nutritional conditions, including nitrogen limitation, carbon source composition, light regime, and redox balance.

We intend to use the liquid fraction of pre-treated barley straw, through HTC at different temperatures and retention times, as carbon substrates in anaerobic digestion by PPB to generate valuable and commercial products such as biogas, biomethane, and mainly, hydrogen, its quality and usability. Ultimately, this work aims to evaluate the suitability of these biomass-derived fractions for supporting PPB growth and hydrogen production under controlled anaerobic conditions. By integrating biomass pretreatment with microbial hydrogen production, we explores a promising strategy for simultaneous waste valorization and renewable energy generation, while contributing to the optimization of PPB-based biohydrogen systems.

Keywords: Biohydrogen, Purple phototrophic bacteria (PPB), Biomass valorization

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BIO-BASED COMPOUNDS FROM *CHLOROFLEXUS AURANTIACUS*: THE ROLE OF LIGHT INTENSITY AS STRESS CONDITION AND CARBON SOURCE

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Recent advances increasingly emphasize the microbial production of value-added bioproducts, including polyhydroxyalkanoates (PHA) and photosynthetic pigments such as bacteriochlorophylls (BChl) and carotenoids, as sustainable alternatives to synthetic chemicals. This study identified cultivation strategies that favour the production of bio-compounds by the thermophilic green non-sulfur bacterium *Chloroflexus aurantiacus* Y-400-fl (DSM 637). Biomass was collected from a photobioreactor, operated at an initial concentration of sulphide of ~10 mg S^{2-} L^{-1}, at 50 ºC under continuous illumination (2.3 ± 1.3 W L^{-1}) and fed with yeast extract either as the sole carbon source or supplemented with glycyl-glycine. 13 batch assays were performed to evaluate the effect of light intensity as a stress condition, along with different carbon-source supplements (yeast extract, glycine, sodium acetate, valeric acid, or sodium carbonate), on pigment and PHA production under anaerobic conditions. For each batch test, three different photoperiods were tested: (i) 24h light, (ii) 6h of light followed by 18h of dark, and (iii) 24h dark.
24h of continuous illumination at light intensities from 2.1 ± 1.4 to 12.3 ± 5.0 W L^{-1} as a stress condition, increased the content of total carotenoids up to 2.3 ± 0.7 % dcw without carbon supplementation. Conversely, under this condition, a detrimental effect on PHA accumulation was observed. The maximum specific growth rate of this strain was observed when yeast extract was supplemented, reaching values of ~1.4 d^{-1}. Sodium acetate, valeric acid, and sodium carbonate supplementation impaired *C. aurantiacus* growth, resulting in negative values of the growth rate. The accumulation of photosynthetic pigments was enhanced when glycine was supplemented, with up to 3 times higher total carotenoid content after 24h of incubation under the three different photoperiods. In addition, an increase in BChls *c* from 2.5 to 3.9 % dcw was observed after 24h dark under sodium carbonate supplementation. These results confirm that photosynthetic pigment production in *C. aurantiacus* can be tuned in response to cultivation conditions. Notably, growth at 50 ºC and the presence of sulfide are unfavorable for most pigment-producing microorganisms, including microalgae. These selective conditions are expected to favor the predominance of *C. aurantiacus* in non-sterilized systems while reducing the costs associated with cooling and sterilization. This opens the possibility of developing new and sustainable bioprocesses for pigment production.

Keywords: Chloroflexus aurantiacus, Light intensity, Photosynthetic pigments, Polyhydroxyalkanoates
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SELECTIVE CULTIVATION OF *ARTHROSPIRA PLATENSIS* USING NH_3 TOLERANCE TO SUPPRESS MICROALGAL AND CYANOBACTERIAL CONTAMINANTS

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Contamination by microalgal and cyanobacterial species remains a persistent challenge in the outdoor cultivation of *Arthrospira platensis*. Free ammonia (NH_3) is known to inhibit the growth of many microalgal species, whereas *A. platensis* is less susceptible to NH_3 inhibition. Taking advantage of its high NH_3 tolerance, this study examined the feasibility of selective cultivation of *A. platensis* under NH_3-rich conditions using cyanobacterium *Synechococcus leopoliensis* as a model contaminant, which shows high NH_3 tolerance same as *A. platensis*. The half-maximal effective concentrations (EC_{50}) of NH_3 were 3.8 ± 0.4 mmol L^{-1} for *A. platensis* and between 1.0 and 2.0 mmol L^{-1} for *S. leopoliensis* in batch monocultures. In the following experiment, the two species were co-cultivated semi-continuously at an NH_3 concentration of 2.5 mmol L^{-1}, which was expected to enable selective cultivation of *A. platensis.* Although the growth rate of *A. platensis* decreased compared with batch monoculture, *A. platensis* maintained a biomass productivity of 9.30 $\times 10^8$ cells L^{-1} day^{-1}, while the growth of *S. leopoliensis* was completely suppressed. These results suggest that NH_3-based selective cultivation of *A. platensis* can suppress a wide range of contaminant species in outdoor cultivation system by appropriately adjusting the NH_3 concentration, since *S. leopoliensis* exhibits relatively high NH_3 tolerance than other microalgal and cyanobacterial species.

Keywords: Cyanobacteria, Arthrospira platensis, Free ammonia, Contamination, Selective culture

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TEMPERATURE EFFECT ON PHOTOSYNTHETIC MEMBRANE ORGANISATION OF PURPLE BACTERIA STUDIED BY ATOMIC FORCE MICROSCOPY

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Purple phototrophic bacteria, due to their relative simplicity, have become one of the most intensively studied photosynthetic organisms. Their photosynthetic apparatus, located in specialised membrane vesicles termed chromatophores, comprises a reaction centre (RC) and one or two types of antenna complexes, called LH1 and LH2, respectively. The structure and function of individual light-harvesting complexes are already well established; however, their molecular organisation and properties in native membranes subjected to environmental changes need further research. Atomic force microscopy (AFM) is a powerful tool that enables information at subcellular resolution. It is a unique technique that can be used for in situ identification of the components of multiprotein assemblies, such as photosynthetic complexes, as well as detailed studies of their supramolecular architecture.

The present study aimed to establish the immobilisation method of dehydrated bacterial membranes for AFM measurements and to examine the influence of elevated temperature on the architecture of chromatophores isolated from *Rhodobacter sphaeroides*. As supports for chromatophores, various types of substrata were tested. The isolated bacterial membranes were characterised by spectroscopic techniques. Changes in the organisation of the chromatophores were investigated at a wide range of temperatures (20-45°C) and monitored on several levels: (1) membrane topographies; (2) mechanical properties; and (3) spectroscopic properties. The results indicate that freshly cleaved mica or glass covered with glutaraldehyde are the best methods for membrane immobilisation for AFM temperature studies. Elevated temperature significantly affects the stability of bacterial membranes, results in changes in their spectroscopic properties, and greatly influences the mechanical properties of membranes.

Keywords: purple bacteria; chromatophore architecture, membrane immobilisation

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BIOHYDROGEN PRODUCTION FROM ORGANIC WASTE IN A SPACE ENVIRONMENT: THE POTENTIAL OF PURPLE PHOTOTROPHIC BACTERIA IN THE BIOMOON PROJECT

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The possibility of establishing permanent human settlements beyond Earth's boundaries is strictly linked to the development of technologies capable of autonomously regenerating essential resources. In extreme environments, where supplies from Earth are limited, energy production must necessarily rely on integrated and sustainable biological systems. In this scenario, the BioMoon project explores the potential of purple non sulphur bacteria (PNSB) for the generation of biohydrogen. The process is based on the recovery of organic waste substrates which, through fermentation, are converted into volatile fatty acids (VFA), representing the optimal carbon source for the metabolism of phototrophic bacteria. However, hydrogen production is strictly dependent on nitrogen-limiting conditions: in this state, the bacteria stop cellular growth and divert the energy from VFAs toward the nitrogenase enzyme, which catalyzes the release of biogenic hydrogen. The present work focuses on the application of two different culture configurations to maximize the efficiency of this enzymatic mechanism. In particular, the research aims to determine whether cellular immobilization can offer competitive advantages over traditional suspension culture, with the intent of improving system stability, biomass protection, and the feasibility of separating the produced gas from the liquid medium. The experimental approach was divided into a biomass optimization phase and a subsequent hydrogen production induction phase. For the immobilized system, the ionotropic gelation technique was used in 2% (w/v) calcium alginate spheres. Both configurations were tested inside a photobioreactor under anoxic conditions. By focusing on both the management of suspended biomass and the dynamics of immobilized systems, the study provides parameters for optimizing the management of PNSB in a lunar environment.

Keywords: Bio-hydrogen, Purple non sulphur bacteria, Ionotropic gelation

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IMMOBILIZED CHLORELLA VULGARIS IN ALGINATE MATRICES FOR SUSTAINABLE FOOD PRODUCTION IN CLOSED-LOOP LIFE SUPPORT SYSTEMS

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In the context of future long-duration space missions, the development of Bioregenerative Life Support Systems (BLSS) has become a technological priority. The primary objective of these systems is to ensure human survival in extraterrestrial environments by minimizing dependence on Earth-supplied provisions. In this scenario, the BioMoon project explores the use of immobilized microalgae as a fundamental biotechnological platform for resource recycling and biomass production in closed systems. The core of this study is based on the cellular immobilization technique: cells of the microalga Chlorella vulgaris are not left free in the liquid but are entrapped in spherical sodium alginate matrices (2% w/v) through an ionotropic gelation process. This configuration was selected to enhance system efficiency, as it facilitates biomass separation from the liquid medium and resolves typical challenges associated with free cultures, such as lengthy and complex cell recovery procedures. The experimental activity utilizes a vertical column photobioreactor (PBR), designed to ensure continuous recirculation of the nutrient medium. This structure ensures homogeneous nutrient distribution and prevents the beads from accumulating at the bottom. To support algal metabolism and ensure optimal mixing, a gas mixture composed of 90% air and 10% CO_2 is injected at the base of the column. The research aims to demonstrate that the system can sustain high cellular viability and constant biomass growth within the gel beads, while also validating the process efficiency in producing high-nutritional-value algal biomass, intended to supplement astronauts' diets as an in situ sustainable food source. The approach proposed by the BioMoon project thus represents a robust, scalable, and fundamental solution for the integration of circular processes, enabling the creation of closed and self-sufficient artificial ecosystems for space exploration.

Keywords: Cellular immobilization, Chlorella vulgaris, microalgae.

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BIOREMEDIATION AND RESOURCE RECOVERY FROM CHEESE WHEY THROUGH PROTEIN-RICH MICROALGAE CULTIVATION

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This study evaluated the feasibility of cultivating protein-rich microalgae using digested cheese whey (DCW) as source of nutrients. Cheese whey is the main byproduct of cheese production, and it poses an environmental challenge when not properly managed. While anaerobic digestion can be performed to remove organic matter of cheese whey and convert it into biogas, the digested effluent retains substantial nutrient loads, requiring further treatment or valorization. In this study, digested cheese whey (DCW) was evaluated as an alternative nutrient source for the cultivation of three microalgae with potential food and feed applications: the marine species *Nannochloropsis gaditana* and *Tetraselmis chuii*, and the freshwater species *Chlorella vulgaris*. Triplicate batch experiments were conducted in 200 mL reactors under three culture conditions for each strain: synthetic medium (control), DCW as sole nutrient source, and a mixed medium combining DCW with synthetic nutrients.

All three strains grew successfully using DCW as a nutrient source, reaching final biomass concentrations between 640 and 910 mg L^{-1} of volatile suspended solids and showing biomass productivities comparable to, and in some cases higher than, those obtained with synthetic media. The culture media did not significantly affect biomass protein content, which ranged from 24.9 to 58.2% of dry weight depending solely on the strain. All three strains were highly effective removing nitrogen, with average efficiencies above 90%. *Chlorella vulgaris* was the fastest out of the three, removing about 40 mgN L^{-1} within only three days; however, *Tetraselmis chuii* displayed the highest specific growth rates, up to 1.6 d^{-1}, as well as a higher and more stable phosphorus removal.

These results demonstrate that DCW can replace synthetic culture media for the simultaneous bioremediation and valorization of cheese whey through the production of protein-rich microalgal biomass, supporting more circular and sustainable dairy waste management strategies.

Keywords: Photorefineries, microalgae, cheese whey, nutrients recovery, single-cell protein.

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CHARACTERIZATION OF RESIDUES FLOWS FROM THE URBAN WASTE TREATMENT PLANT IN WESTERN MACEDONIA LOOKING FOR BIOBASED SOLUTIONS

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Abstract

Waste Management of Western Macedonia (DIADYMA S.A.), founded in 1998, is the Waste Management Agency (FODSA) of the Region of Western Macedonia. The main objective of the company is the design, implementation, and operation of the regional Integrated Waste Management System (IWMS) of Western Macedonia, with the implementation of sustainable management methods. The Mechanical and Biological Treatment plant (MBT), consisting the first Private-Public-Procurement (PPP) project in Greece, is of great importance within the IWMS of Western Macedonia. Its commercial operation started on 2017 and enabled DIADYMA to ensure the treatment of all waste generated in Western Macedonia. This plant has a max. capacity of 120,000 tn/y and includes mechanical sorting of mixed waste into recyclable and organic, followed by mechanical treatment and separation of recyclable materials in streams with wide use of optical, gravitational, ballistic separators, as well as manual sorting, while the organic fraction is subjected to aerobic processing and composting under controlled conditions. The MBT plant flowchart is given in Figure 1.

The composition analysis in the inlet of the MBT plant has showed that the organic fraction consists around 40% of the total waste amounts received, the recyclable fraction is around 35.5%, with the rest being divided into materials unable to be recovered within the separation process (~15%), tires/leather/textile/wood (~7.5%) and inert materials (~2%). Based on recorded mass balances, the biodegradable fraction, consisting of the organic fraction and mixed paper, diverted from landfilling reaches almost 91%, the Recyclable materials recovery yield in the Mechanical Separation process is around 49%, while the total residual flow, including bulky residues, mechanical separation residues and refinery residues, ending to landfill reaches around 35%.

The aim of the present study was the characterization of the solid rejects resulting from the Mechanical Separation Unit as well as after composting process (Refinery Unit residues) before the final product is obtained, to increase the knowledge about their composition. Therefore, the flows will be characterized based on total and volatile solids, ash, moisture, and elemental composition (C, H, N, O, S, P). Final goal is to evaluate the possible valorization of these residues, which end to landfill, as raw material for the combined pyrolysis and gasification process, obtaining high-value added products, such as biochar and syngas. Moreover, the Compost-Like Output (CLO Type A) resulting from the composting process, which is up to now used for soil restoration in the region, will be examined.

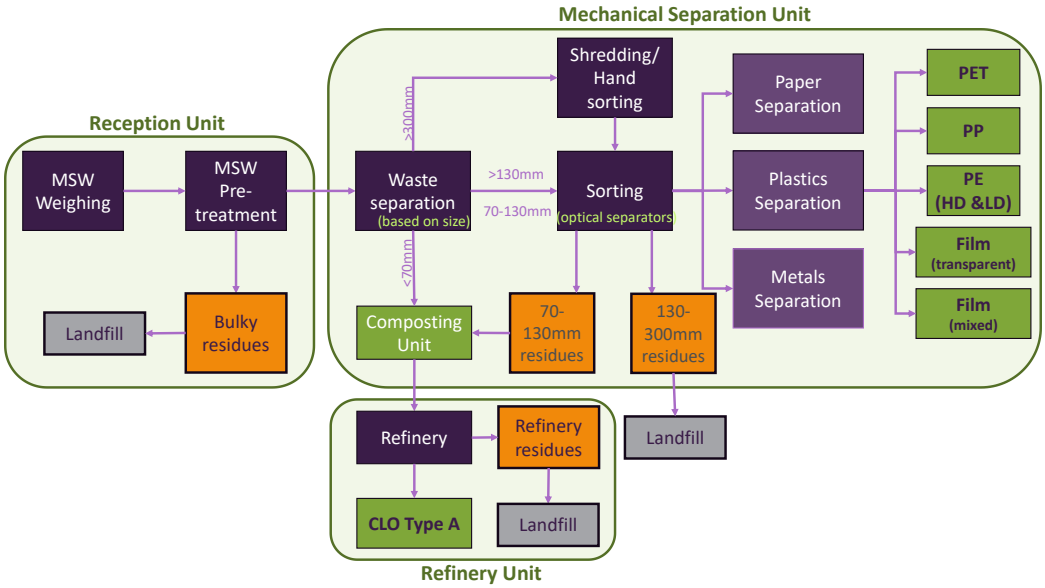


Figure 1. MBT plant flowchart.

Materials and methods

The sample size was defined to obtain representative data and designed according to the diverse flows of MBT plant. The flows selected to be analyzed are shown in Figure 2 and were i) Mechanical Separation Unit - residues removal, ii) Refinery Unit – residues removal, iii) Compost-Like Output (CLO Type A). The sampling process was performed to ensure homogenized conditions in each sample, considering the level of variation of the residues in each flow.

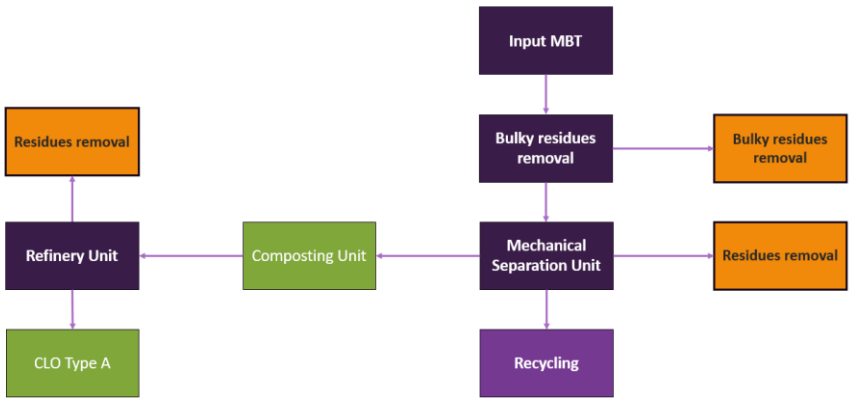


Figure 2. MBT plant flowchart.

Characterization of samples

Moisture, total solids (TS), volatile solids (VS), and ashes were calculated following the CEN/TS 15414-1 and EN 15403 methods. This principle is based on weight loss at a specific time and temperature of a sample.

The pH values were determined using the EPA's 9045D method (9045D, Environmental Protection Agency). The measurement was made using a pH meter (Phoenix instrument, EC-26 pH). Total nitrogen content was determined following the Kjeldahl method (APHA Standard Methods 4500-Norg B). Elemental analysis was performed by using a Thermo Fisher Scientific FlashSmart™ Elemental Analyzer according to EN 15407:2011 method.

Keywords: waste management, mechanical separation unit residues, refinery unit residues, valorization.

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STUDIES OF EXTRACELLULAR ELECTRON TRANSFER IN *RHODOPSEUDOMONAS PALUSTRIS* TIE-1 AIMING TO IMPROVE BIOELECTROCHEMICAL APPLICATIONS

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Microorganisms living in oxygen-limited environments have evolved diverse strategies to sustain energy metabolism. Among these, extracellular electron transfer (EET) enables certain anaerobic microbes to exchange electrons with insoluble electron acceptors such as minerals or electrodes, effectively extending their respiratory network to the outside surface of the cell. The metabolically versatile purple bacterium *Rhodopseudomonas palustris* TIE-1 is well-characterized for its ability to accept electrons from solid donors (microbial electrosynthesis). However, its capacity to function in the reverse direction, donating electrons to an anode during anaerobic respiration, has only recently been observed, and the underlying molecular conduits remain uncharacterized.

We hypothesize that the outer membrane porin cytochrome complex PioAB is a primary mediator of this outward electron flux. The complex consists of the decaheme c-type cytochrome PioA inserted into the β-barrel protein PioB, the complex is structurally positioned to bridge the periplasm and the extracellular environment. To characterize its function in electron donation, we performed its detailed molecular characterization. The PioAB complex was successfully cloned and purified, enabling the evaluation of its redox properties by a combination of protein film voltammetry and NMR spectroscopy.

In vivo, active current production by TIE-1 was quantified in a bioelectrochemical system poised at 200 mV vs. Ag/AgCl. Confocal microscopy of the anodes confirmed direct bacterial attachment, indicating a direct rather than mediated electron transfer mechanism. To explicitly link the PioAB complex to this electricity production, we are evaluating the electrochemical performance of a wild-type strain against a ΔpioAB deletion mutant. Collectively, this work aims to elucidate the structural and functional basis of outward EET in *R. palustris* TIE-1, expanding our understanding of how these metabolically flexible microbes exploit alternative respiratory pathways in anoxic environments.

Keywords: Rhodopseudomonas palustris TIE-1, Extracellular electron transfer, Bioelectrochemical system.

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TRANSCRIPTOMIC CHARACTERIZATION OF *RHODOPSEUDOMONAS PALUSTRIS* 42OL IN BIO-ELECTROCHEMICAL SYSTEMS FOR OPTIMIZED RESOURCE RECOVERY

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Rising operational costs and an increased environmental footprint in the agri-food sector have intensified the search for sustainable waste-to-energy technologies. Although high-COD (Chemical Oxygen Demand) food processing residues are difficult to manage, they offer a significant opportunity for resource recovery through bio-electrochemical systems (BES). Purple non-sulfur bacteria, particularly *Rhodopseudomonas palustris*, are ideal for this application thanks to their metabolic flexibility. When integrated into photo-bio-cathodes, these microorganisms can theoretically use cathode-derived electrons to synthesize biomass and bio-hydrogen. However, the overall efficiency of BES is often limited by the slow kinetics of extracellular electron transfer (EET) at the microbe-electrode interface. Previous results using *R. palustris* 42OL demonstrated a significant metabolic shift when anthraquinone-2,6-disulfonate (AQDS) was utilized as a redox mediator in a BES polarized at -0.6 V (*vs* Ag/AgCl). While mediator-free controls prioritized growth (OD_{660} = 2.56 ± 0.01), the addition of AQDS under cathodic polarization triggered a 17-fold increase in cumulative charge recovery (142.70 ± 40.20 C) and prioritized H_2 production (141.93 ± 7.71 mL). This inverse relationship suggests that AQDS acts as a metabolic switch, redirecting electrons to bio-H_2 synthesis. To elucidate the molecular mechanisms underlying this redirection, we have designed a comparative transcriptomic study focusing on the exponential growth phase of *R. palustris* 42OL. A unique experimental setup will be employed in which cathodic polarization (-0.6 V *vs* Ag/AgCl) is cycled "on" and "off" within the same culture to isolate the immediate transcriptional response to electrical stimuli. This will be done by comparing two different conditions: with and without the redox mediator AQDS. High-resolution long-read sequencing via the Oxford Nanopore PromethION platform will be used to identify differential gene expression and operon structures. The study will focus on quantifying the regulation of genes involved in nitrogenase/hydrogenase complexes, the Calvin–Benson–Bassham (CBB) cycle, and the EET machinery of this specific strain. By correlating these transcriptomic profiles with electrochemical performance, the study will provide a molecular blueprint for optimized resource recovery in photo-bio-cathodes.

Keywords: Purple Phototrophic Bacteria, Transcriptomics, Bio-electrochemical Systems, Resource Recovery, mRNA-seq

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PHOTOGRANULE PERFORMANCE UNDER OPTICAL LIMITATION IN REAL WASTEWATER STREAMS

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Photogranules have demonstrated high efficiency performance in synthetic media and clarified primary effluent; however, their behavior under cultivation in real secondary effluent with high turbidity, dissolved organic matter, and complex carbon remains poorly understood. This study evaluated photogranule performance under optically limiting conditions using a 2×2 factorial design in four sequencing batch reactors (SBR) operated for 52 days. Two wastewater types were tested: sanitary UASB effluent (~40 NTU; L1, L2) and a mixture of UASB effluent with anaerobically digested animal manure (~400 NTU; H1, H2). Two hydraulic retention times (HRT) were applied (1.0 and 1.7 d). Photogranules remained structurally stable across all conditions, achieving separation efficiencies above 98% and SVI below 60 mL g^{-1} from day 26 onwards, indicating neither turbidity nor carbon complexity impaired granule stability. Despite increased light attenuation in high-turbidity reactors (OD_{680} ≈ 0.12–0.14 vs 0.05–0.09), dissolved oxygen concentrations remained high (6.9–7.4 $mg.L^{-1}$), confirming sustained photosynthetic activity under light-limited conditions. Wastewater composition influenced extracellular polymeric substances (EPS) production, with higher accumulation in manure-loaded reactors (~205–237 $mg.L^{-1}$) compared to sanitary wastewater (~104–168 $mg.L^{-1}$). Nitrogen transformation pathways were strongly affected by both HRT and organic loading. In sanitary wastewater, only a small fraction of influent NH_4^+-N was recovered as NO_3^--N in the effluent (<10%), whereas in hybrid wastewater, nitrate accumulation was substantial at shorter HRT (~80% of influent NH_4^+-N at 1.0 d) and decreased markedly at longer HRT (~17% at 1.7 d). The rapid response of nitrate concentrations to HRT changes indicates that hydraulic retention time is a key operational driver of nitrogen transformation. The contrast between sanitary and hybrid wastewater highlights the combined influence of organic loading and wastewater composition, suggesting a shift from phototrophic assimilation towards nitrification under elevated COD:NH_4^+ ratios. These findings demonstrate that photogranules are structurally resilient under optically limiting conditions and that operational parameters, particularly HRT and organic loading, govern nitrogen pathways in complex real wastewater.

Keywords: Photogranules, Optical stress, Anaerobic animal Manure, Wastewater treatment

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INVESTIGATION OF THE EFFECTS OF *RHODOBACTER CAPSULATUS* AND *CEREIBACTER SPHAEROIDES* ON THE GROWTH OF *CALENDULA OFFICINALIS* ON NICKEL-CONTAMINATED SOIL

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Heavy metal contamination represents a major environmental concern due to its detrimental effects on soil fertility and plant development. In recent years, biological remediation approaches have gained increasing attention as sustainable strategies for mitigating heavy metal pollution. In this study, the effects of *Rhodobacter capsulatus* and *Cereibacter sphaeroides* on plant growth and nickel phytoremediation capacity of the medicinal and aromatic plant *Calendula officinalis* were investigated.

Seeds of *C. officinalis* were initially germinated in peat and subsequently transplanted into pots. The experiment was conducted with three replicates and consisted of four treatments: control, 25 mg kg^{-1} Ni, 25 mg kg^{-1} Ni + *R. capsulatus*, and 25 mg kg^{-1} Ni + *C. sphaeroides*. Each pot contained three plants, and bacterial suspensions were inoculated into the rhizosphere. The experiment was maintained under controlled laboratory conditions for two months. Following harvest, elemental analyses of plant tissues, roots, and soil samples were performed using inductively coupled plasma–optical emission spectrometry (ICP–OES). Ethyl acetate extracts were prepared from plant materials, and their antibacterial activities were evaluated against selected bacterial strains.

The results indicated that bacterial inoculation mitigated the adverse effects of nickel stress on plant development. *C. sphaeroides* significantly enhanced plant growth under nickel exposure, whereas *R. capsulatus* promoted root development. Nickel accumulation in plant tissues was associated with a reduction in antibacterial activity; however, bacterial inoculation partially restored this activity. Overall, the findings demonstrate the beneficial effects of purple non-sulfur bacteria on plant performance and stress tolerance during the cultivation of medicinal and aromatic plants in nickel-contaminated soils. Furthermore, the results suggest that *C. officinalis* may serve as a potential accumulator species suitable for use in phytoextraction-based phytoremediation strategies.

Keywords: Purple non sulfur bacteria, soil contamination, heavy metal, bioremediation

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EXCITON AND ELECTRON TRANSFER IN BACTERIAL PHOTOSYNTHETIC UNIT

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Purple phototropic bacteria's photosynthetic complex transports light in the harvesting complex and electrons in the reaction center. Primary charge separation in the reaction center runs with ultrafast speed and transforms light into chemical energy with high efficiency. We show that electrons are directed by the vibration modes into the needed site where they are localised [1]. We describe the exciton transport between the light-harvesting complex LH1 and the reaction center dimer. We also describe the hot electron transfer(ET). This type of ET can be used in the bacterial reaction centers. The electron-hole separation is more efficient in this case. The result is high productivity of chemical energy by solar energy in biological systems[2].

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EVALUATION OF WASTEWATER FROM CLOSED-SYSTEM TILAPIA FARMING AS A GROWTH MEDIUM FOR SPIRULINA

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Due to the growing demand for animal protein and the decline in natural stocks caused by overfishing, numerous fish farms have been established in recent years; tilapia farming is now practiced in more than 140 countries worldwide and has become a significant industry. This rapidly expanding aquaculture industry generates wastewater, and the discharge of this contaminated water into the environment contributes to eutrophication, a major problem in natural water bodies. The wastewater in aquaculture contains chemicals, feed, pathogens, and various microorganisms, and this wastewater contains solid matter in both suspended and dissolved forms. The use of this wastewater, which has a high organic load, in microalgae cultivation is emerging as an economically advantageous practice. In this study, we investigated the use of wastewater generated from a closed-system tilapia (*Oreochromis niloticus*) aquaculture system for the cultivation of *Arthrospira platensis* in batch reactors. The three conditions in the experiment were control (100% Zarrouk medium prepared with filtered tap water), 25% of the medium replaced by wastewater with or without UV treatment for 120 min. Daily growth of Spirulina was followed spectrophotometrically. After the harvest, chlorophyll, carotenoid, phycocyanin contents were measured. The presence of pathogens in wastewater decreased the yield of microalgae. Replacing 25% of Zarrouk medium with tilapia wastewater decreased the total chlorophyll content of *A. spirulina*, however, carotenoid and phycocyanin contents of the group with UV treated wastewater was almost the same as the control condition. The latter two bioactive compounds are economically very valuable, hence the use of aquaculture wastewater, with a prior treatment to eliminate pathogens, can be safely used for *A. spirulina* cultivation. This approach can also help reduce the costs associated with the use of nutrients in the production of microalgae, such as spirulina, from wastewater generated in closed-system aquaculture.

Keywords: Tilapia, Wastewater, A. platensis, Bioactive molecules

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AI-ASSİSTED AND MODULAR PHOTOREACTOR DESİGN FOR SUSTAİNABLE RESOURCE RECOVERY: CHALLENGES AND FUTURE PERSPECTİVES

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Photorefinery technologies have emerged as promising sustainable platforms for converting waste streams, biomass, carbon dioxide, and industrial effluents into value-added products through light-driven processes. However, the large-scale implementation of photoreactors remains limited due to challenges associated with light penetration, energy efficiency, mass transfer limitations, photocatalyst stability, reactor fouling, and process scalability. In addition, non-uniform photon distribution and high operational costs continue to restrict industrial adaptation of current photoreactor systems. This study reviews recent advances in photoreactor design with a particular focus on modular and AI-assisted reactor configurations for resource recovery applications. Different reactor geometries, including tubular, flat-plate, slurry, immobilized catalyst, and microchannel photoreactors, are comparatively evaluated in terms of photon utilization efficiency, hydrodynamics, scalability, and sustainability performance. Furthermore, the integration of computational fluid dynamics (CFD), machine learning, and digital twin technologies for real-time optimization of photoreactor operation is critically discussed. Special attention is given to future photoreactor concepts utilizing renewable energy integration, smart sensor systems, adaptive light management, and hybrid photocatalytic-biological platforms for enhanced circular economy applications. The findings indicate that next-generation photoreactors should prioritize modularity, energy optimization, process intensification, and intelligent control systems to achieve economically feasible and environmentally sustainable resource recovery processes. This work highlights the transition from conventional photoreactor systems toward smart and adaptive photorefineries capable of supporting future low-carbon and circular bioeconomy strategies.

Keywords: Photoreactor design; Photorefineries; Resource recovery; Photocatalysis; Artificial intelligence; Computational fluid dynamics (CFD)

BEYOND REMEDIATION: TECHNICAL READINESS AND ECONOMIC FRONTIERS OF PHOTOTROPHIC PURPLE BACTERIA (PPB) BIOREFINERIES (2024–2026)

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Since 2024, Phototrophic Purple Bacteria (PPB) technology has transitioned from fundamental metabolic studies to integrated pilot-scale validation, marking a significant increase in its Technology Readiness Level (TRL), currently estimated at TRL 5–7 for specific applications like domestic wastewater treatment and nutrient recovery. A review of over 50 recent scientific articles indicates that contemporary research is increasingly centered on the metabolic engineering of purple non-sulfur bacteria to optimize high-value outputs. Key trends include the use of specific infrared (IR) light tuning to achieve biomass containing up to 49% Polyhydroxyalkanoates (PHA) and optimized denitrification processes that achieve nitrate removal rates of 0.68 mg N/L/h with minimal nitrous oxide emissions. Furthermore, the period has seen the emergence of PPB as a cornerstone for single-cell protein (SCP) and pigment production (e.g., carotenoids) for the aquaculture industry.

Despite these advances, significant challenges remain, including the high costs associated with biomass harvesting, the need for large-scale photobioreactor optimization to ensure uniform light distribution, and the complexity of maintaining stable mixed-culture populations in non-sterile industrial environments. Future perspectives point toward the integration of PPB into "photo-biorefinery" clusters where wastewater serves as a sustainable feedstock for multiple revenue streams, including biohydrogen (H_2) and bioplastics. Emerging studies are also exploring the use of PPB in bioelectrochemical systems to increase production rates two to three-fold compared to conventional reactors. As the technology approaches economic competitiveness with petroleum-based plastics, the next phase of development (2026–2030) will likely focus on full-scale commercial deployment and the standardization of PPB-derived biofertilizers.

REDOX BALANCING MECHANISM IN PURPLE BACTERIA: FROM MACROMOLECULES TO METABOLIC GENE EXPRESSION

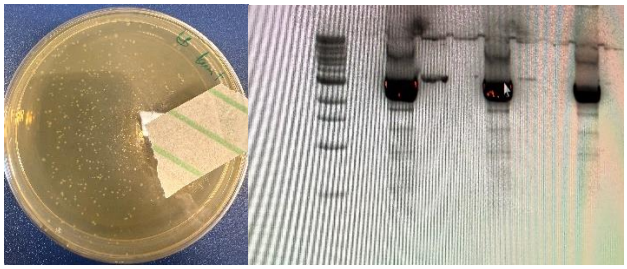
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Purple non-sulfur bacteria (PNSB) are photoheterotrophic microbes known for their metabolic versatility and growing use in environmental and Food biotechnology. They maintain redox homeostasis through various electron-sinking strategies, particularly under anaerobic, VFA-rich conditions. This study explores redox regulation in different PNSB strains by analyzing macromolecular composition, isoleucine accumulation, and the role of the isocitrate lyase (ICL) pathway.

Four PNSB strains(*Rs.rubrum, Ce.sphaeroides, Rh.capsulatus, R.palustris*)were cultured under photoheterotrophic conditions with propionate and butyrate in the presence of 50 mM sodium bicarbonate (Cabecas-Segura et al 2021). Biomass was analysed for macromolecular composition, including protein, lipid, carbohydrate, nucleic acid, PHA, and ash content. Isoleucine levels were quantified by LC-MS (ZenoTof7600) in *Rs.rubrum and R. palustris grown under* acetate and butyrate conditions. The aceA and aceB genes from *Rh.capsulatus* were cloned into a plasmid under the puf promoter and transformed into *Rs.rubrum* for heterologous isocitrate lyase expression, followed by PCR/plasmid confirmation and metabolic characterization.

PNSB showed substrate-dependent redox balancing, with strain-specific changes in biomass composition under different carbon sources. Differential isoleucine accumulation in *Rs.rubrum* and *R.palustris* during growth on acetate and butyrate suggests distinct metabolic redox responses. Additionally, the aceA and aceB genes from Rh.capsulatus were successfully cloned and verified, and the construct is ready for transfer into Rs.rubrum for metabolic characterization.



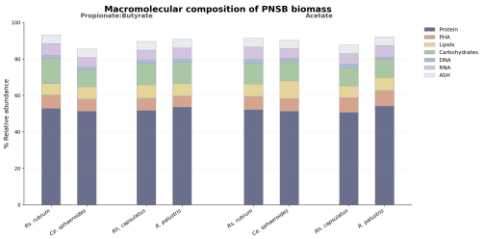


Figure 1a&b: Transformed plate of the Puf plasmid and PCR confirmation of aceA and aceB genes.

Figure 2: Ratios of macromolecules in biomass using selected biochemical assays.

Keywords: Purple Non-Sulfur Bacteria, Photoheterotrophic bacteria, Redox homeostasis, Volatile fatty acid, electron sink

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HIGH-THROUGHPUT ENRICHMENT OF HALOALKALIPHILIC PURPLE BACTERIA FOR MICROBIAL FOOD APPLICATIONS

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Meeting future protein and micronutrient demand requires scalable, resource-efficient microbial platforms. Purple phototrophic bacteria (PPB) produce protein-rich biomass together with antioxidants such as carotenoids and coenzyme Q10, and can also supply essential vitamins and minerals. Haloalkaliphilic PPB (HA-PPB) are particularly suited for food-grade production: tolerance to up to 3 M NaCl and pH 12 enables non-sterile operation, lowers freshwater demand, and reduces contamination risk. Despite this potential, HA-PPB remain largely underexplored.

This study presents, to our knowledge, the first high-throughput enrichment of anaerobic phototrophic communities. Environmental samples from Gruissan and Peyriac-de-Mer (Narbonne, France) and collected in the Rio Tejo estuary (Samouco, Portugal) haloalkaliphilic habitats were enriched in a BioLector XT microbioreactor under near-infrared light (805 nm). Anaerobic batch assays in 48-well plates tested a factorial range of pH (6.8–12.0) and salinity (1–105 g L^{-1} NaCl), with online backscatter (625 nm) used as a proxy for biomass growth.

All three environmental samples yielded robust anoxygenic phototrophic enrichments across a range of pH and salinity conditions. At seawater salinity (35 g L^{-1} NaCl), pH 10.0 consistently gave the strongest growth, reaching ~900 a.u. by 70 h; pH 6.8 and 8.0 achieved similar yields but with slower kinetics. At higher salinities (70–105 g L^{-1} NaCl), pH 10.0 remained optimal, with longer lag phases (~30–40 h) but comparable final biomass (~850–1000 a.u.), indicating clear halotolerance. Growth at pH 12.0 was largely inhibited, except for one Portuguese sample that showed growth at low salinity (1 g L^{-1} NaCl), suggesting the presence of highly alkaliphilic taxa absent in the French samples.

This work establishes a high-throughput framework to discover HA-PPB with improved antioxidant potential. The enriched communities provide a basis for targeted isolation and detailed genomic and macromolecular characterization (ongoing). These next steps will support the development of HA-PPB as a scalable microbial platform for protein and bioactive production.

Keywords: halo-alkaliphilic purple phototrophic bacteria; high-throughput enrichment; BioLector XT .

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DIFFUSE GAS FLOWS CONVERSION INTO HIGH-VALUE-ADDED COMPOUNDS BY THERMOPHILIC PHOTOTROPHIC BACTERIA IN THE POST-PURPLE PROJECT

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Waste treatment is an essential part of the EU's transition towards a circular economy in the coming years. Nowadays, the waste treatment sector is responsible for large emissions of gases such as CO, CO_2, NO_x, CH_4, VOCs, and NH_3. These gases are emitted to the atmosphere through direct and diffuse emissions or treated in conventional purification plants, generating a new residue. The POST-PURPLE project aims to contribute to the zero-waste goal by developing innovative bio-based technologies to achieve the full valorisation potential of urban biorefinery waste, allowing for the production of high-value products like carotenoids, coenzyme Q10, vitamin B12, and microbial protein while reducing diffuse gas emissions and optimising the treatment of wastewater and of the organic fraction of municipal solid waste.

In this project, URJC, in collaboration with the ISP, will develop a novel technology based on the use of thermophilic purple phototrophic bacteria (PPB) for the treatment of syngas produced during waste pyrolysis/gasification and of the condensed vapours generated during the thermal hydrolysis of urban organic waste. This technology will transform syngas into PPB-microbial protein, carotenoids, coenzyme Q10, and vitamin B12, using the condensed vapours as an ammonium source. Firstly, a series of critical factors, such as $CO:H_2$ volumetric ratio, hydraulic retention time, temperature, and irradiance, will be explored in batch tests to determine their effect on project performance, which will be measured as carbon conversion efficiency, specific substrate consumption, biomass, and coenzyme Q10 and vitamin B12 yields. Once the process is developed at laboratory scale, it will be validated at pilot scale in closed tubular photobioreactors in continuous operation. Finally, a mechanistic model explaining the process will be developed using batch experimental data for model calibration and validated with results from continuous pilot-scale operation. This model will enable improved process control and the optimisation of operational strategies.

Keywords: Purple phototrophic bacteria, Syngas, Circular economy, Biorefineries, Photo-biorefineries.

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COMPARATIVE STUDY OF Ag- AND Cu-MODIFIED g-C_3N_4 PHOTOCATALYSTS FOR VISIBLE-LIGHT Cr(VI) REDUCTION

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One of the major toxic heavy metals frequently detected in industrial wastewater is Cr(VI). Among various treatment methods, solar photocatalysis has emerged as a green and efficient technology for reducing Cr(VI) to less harmful Cr(III). g-C_3N_4 (CN) has attracted considerable attention due to its stability under acidic conditions, visible-light activity, and suitable conduction band potential for Cr(VI) reduction. However, the practical application of pristine CN is limited by the rapid recombination of photogenerated charge carriers. Constructing metal/CN (M/CN) heterostructures is an effective strategy for improving visible-light absorption via the localized surface plasmon resonance (SPR) effect of metal NPs and enhancing charge separation through Schottky barrier formation. Nevertheless, insufficient interfacial stability may hinder charge transfer between the components and reduce photocatalytic activity. Therefore, morphology regulation and surface treatment of CN are important for optimizing the number and quality of active sites, while promoting the formation of stable metal/CN heterojunctions.

In this work, pristine CN was synthesized via thermal polymerization of urea, while sulfuric acid treatment was employed to modify its surface properties. Ag or Cu species were subsequently deposited onto pristine and acid-treated CN through chemical reduction of $AgNO_3$ or $CuCl_2$ using $NaBH_4$. A comparative study of noble metal (Ag) and transition metal (Cu) deposition on pristine and modified CN was performed. The photocatalysts were characterized by XRD, FTIR, FESEM, EDS, XPS, PL, DRS, EIS, and TPCR analyses. EDS results revealed higher Ag and Cu content on acid-treated CN, likely due to oxygen-containing functional groups on the modified surface. PL, EIS, and TPCR analyses indicated improved charge carrier separation and reduced recombination in the modified samples. However, XPS analysis showed that identical synthesis conditions led to the formation of metallic Ag NPs, whereas Cu was mainly present in higher oxidation states. Hence, Ag-based samples exhibited superior activity toward Cr(VI) reduction compared to Cu-based, due to the greater stability of Ag, while Cu species undergo oxidation that prevents the formation of well-defined Cu NPs. Consequently, Ag NPs enhance photocatalytic activity through the SPR effect, improved light absorption, and efficient charge carrier separation.

Keywords: photocatalysis, Cr(VI), metal deposition, co-catalysis, nanoparticles;

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BACTERIAL ADHESION AND PHOTOCATALYTIC SURFACES

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Surface can promote or inhibit microbial adhesion. The promotion and inhibition ability can be modified by surface coatings. The photocatalytic surfaces are especially promising for different types of technologies.

Before considering adhesion properties, surfaces need to be characterized. 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, hydrophobicity and surface charge of surfaces substantially impact bacterial colonization of surfaces. Surface properties impact photocatalytic processes.

Keywords: photocatalytic surfaces, bacterial adhesion

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INFRARED LIGHT INTENSITY MODULATES CAROTENOID ACCUMULATION IN A NEWLY ISOLATED *RHODOBACTER* STRAIN

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Purple phototrophic bacteria photosynthetic apparatus contains bacteriochlorophylls and carotenoids, which contribute to light harvesting, energy transfer and photoprotection. Infrared light intensity is an important process variable for directing growth and pigment accumulation. Previous studies have shown that light intensity can regulate both growth and photopigment content in purple phototrophic bacteria, with light limitation at low intensity and possible photoinhibition or stress responses at excessive irradiance.

In this study, a purple phototrophic isolate was obtained using a Winogradsky column prepared from material collected from the bank of the Vardar River in the Skopje region, North Macedonia. Based on absorption spectra and morphological characteristics, the isolate was tentatively assigned to the genus Rhodobacter. Cultures were grown in a custom photobioreactor equipped with green, orange and infrared light sources. Green and orange light were maintained at 100 and 25 μmol photons m^{-2} s^{-1}, respectively, while infrared light intensity was varied at 30, 70, 90 and 100 μmol photons m^{-2} s^{-1}. Growth was monitored by optical density, while extracted pigments were analyzed by spectral scanning and high-performance liquid chromatography with diode-array detection.

Infrared light intensity strongly affected the pigment profile of the isolate. The highest total carotenoid concentration was obtained at 70 μmol photons m^{-2} s^{-1}, where both spheroidenone and spheroidene-related carotenoids accumulated more strongly than under the other tested light conditions. Compared with 30 μmol photons m^{-2} s^{-1}, the total carotenoid concentration increased by approximately 40%, indicating that moderate infrared illumination stimulated carotenoid accumulation. In contrast, carotenoid concentration decreased markedly at 90 μmol photons m^{-2} s^{-1} and remained lower at 100 μmol photons m^{-2} s^{-1}, suggesting that increased infrared intensity beyond the apparent optimum did not proportionally enhance carotenoid production. The relative contribution of spheroidenone increased under the highest infrared intensity, indicating a possible shift in carotenoid composition under high-light stress. The bacteriochlorophyll-related signal showed a different trend, being highest at 30 μmol photons m^{-2} s^{-1} and decreasing at higher infrared intensities, which suggests distinct regulation of carotenoid and bacteriochlorophyll pools. These preliminary results indicate that moderate infrared illumination favors carotenoid accumulation in the strain, while higher intensities may trigger pigment remodeling rather than increased total pigment production.

Keywords: carotenoid accumulation; infrared light intensity

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SPATIAL PROFILING AND SAMPLING PROTOCOL VALIDATION IN A PPB-POWERED RACEWAY POND TREATING MUNICIPAL WASTEWATER

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The transition of municipal wastewater treatment towards resource recovery can be effectively driven by Purple Phototrophic Bacteria (PPB) cultivated in open raceway ponds. However, the physical design of raceway ponds combined with the reduced paddlewheel velocity needed for PPB dominance, can create hydrodynamic heterogeneities. This raises concerns regarding potential dead zones, and uneven microbial distribution, similar to challenges observed in algal phototropic systems. Therefore, standardizing sampling locations is critical to ensure extracted data accurately represents the bulk bioreactor community without spatial bias during system optimization. To address this, a spatial protocol was developed to validate sampling strategies and conduct a preliminary microbial community investigation. The study was conducted at the FCC Aqualia wastewater treatment plant facility in Badajoz; specifically in a raceway pond culturing a community enriched in PPB. Biomass was sampled at the start and at the end of a 2-day batch period from four distinct coordinates: the surface (A) and bottom (B) of the central corridor, and the external (C) and internal (D) bottom portions of the curve beyond the semicircular deflector. In order to validate the protocol, 16S and 18S rRNA amplicon sequencing was conducted to map the community across these four spatial points. Concurrently, chemical profiling demonstrated highly successful Chemical Oxygen Demand (COD) and phosphorus removal over the operational period. Total COD and total phosphate dropped to 19.4 mg/L and 0.115 mg/L respectively, easily meeting stringent European Union discharge limits. However, total nitrogen reduction was limited, with effluent concentrations remaining at 17.6 mg/L. This incomplete nitrogen assimilation highlights the critical need to optimize operational parameters. Consequently, establishing this validated spatial sampling protocol is a foundational prerequisite for accurately mapping PPB metabolic networks and improving simultaneous nutrient recovery in subsequent continuous-mode operations.

Keywords: Microbial Consortia, Wastewater Valorization, Biorefinery, Photobioreactor Optimization

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WHITE LIGHT CULTIVATION OF *CYCLOTELLA CHOCTAWHATCHEANA* FOR EPS PRODUCTION AND APPLICATION IN CHITOSAN-BASED FILMS

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Microalgae are promising sustainable cell factories for the production of biomass and extracellular polymers with potential applications in bio-based materials. In this study, *Cyclotella choctawhatcheana* was cultivated under white light in saline f/2 medium to evaluate biomass growth and the production of an EPS-enriched extracellular fraction for use in chitosan-based films. Over a 30-day cultivation period, growth was monitored by optical density, while pH variation and nutrient consumption in the culture medium were also assessed. White light supported effective biomass accumulation and promoted the recovery of an extracellular fraction by ethanol precipitation. This EPS-enriched fraction was then incorporated into chitosan films, leading to measurable changes in key physicochemical properties, including thickness, opacity, moisture content, and water solubility, when compared with the control film. These results indicate that *C. choctawhatcheana* cultivated under white light can serve as a sustainable source of EPS-rich material for the development of functional biobased films. Overall, this work supports the valorization of microalgal biomass through a simple cultivation strategy and highlights the potential of microalgal EPS as renewable additives for biodegradable packaging materials.

MICROALGAL EXOPOLYSACCHARIDES AS SUSTAINABLE FUNCTIONAL INGREDIENTS FOR BIODEGRADABLE FILMS: PRODUCTION, PROPERTIES AND APPLICATIONS

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Microalgal exopolysaccharides (EPS) have emerged as promising sustainable biomolecules for the development of biodegradable films and other bio-based materials. Produced as extracellular carbohydrate-rich polymers, these compounds exhibit considerable structural diversity, which translates into distinct physicochemical properties such as viscosity, water retention, film-forming ability, and bioactivity. Their production is strongly influenced by cultivation conditions, including light quality, salinity, nutrient availability, and stress exposure, which can modulate both biomass productivity and EPS secretion. In recent years, increasing attention has been given to the use of microalgal EPS as functional ingredients in biodegradable packaging materials, where they may act as reinforcing agents, plasticizers, or bioactive additives that improve film performance. This review summarizes the current knowledge on microalgal EPS production, composition, and functional properties, with particular emphasis on their integration into biodegradable films. It also discusses the main challenges associated with upstream production, downstream recovery, and process scalability, as well as the opportunities offered by microalgae-based biorefineries for the valorization of renewable biomass. Overall, microalgal EPS represent a versatile and sustainable resource with strong potential to contribute to the development of next-generation biodegradable materials.

CAS9-ENABLED SINGLE-CELL ANALYSIS REVEALS NITROGENASE EXPRESSION HETEROGENEITY IN *RHODOSPIRILLUM RUBRUM*

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Microbial processes are commonly described using population-averaged measurements, potentially masking phenotypic heterogeneity that can influence metabolic activity and stability. In purple phototrophic bacteria (PPB), this limitation is critical for hydrogen production, as nitrogenase activity is tightly regulated and may vary between individual cells. Understanding this heterogeneity is therefore essential to better describe and control PPB physiology. Here, we investigated nitrogenase expression at the single-cell level in *Rhodospirillum rubrum* S1H using a genome-integrated fluorescent reporter. A *nifK::DsRed* strain was constructed via Cas9-mediated insertion of the DsRed coding sequence at the C-terminus of the chromosomal *nifK* gene, preserving native operon regulation (Figure 1). This approach enables direct, single-cell visualization of nitrogenase expression while maintaining physiological control of the operon. Epifluorescence microscopy revealed strongly condition-dependent expression patterns. Under diazotrophic conditions with a propionate-butyrate mixture, nitrogenase expression was homogeneous across the population (Figure 2A). In contrast, under ammonium-replete conditions, expression was largely repressed, yet rare fluorescent cells were consistently observed during growth on succinate (Figure 2B), and more prominently on acetate (Figure 2C) under additional light stress. No fluorescence was detected in the rich control medium (Figure 2D). These observations demonstrate that nitrogenase expression in *R. rubrum* is not strictly homogeneous and can give rise to metabolically distinct subpopulations under specific environmental conditions. The higher frequency of fluorescent cells under acetate compared to succinate indicates that increased redox pressure, further enhanced by light stress, drives partial derepression of nitrogenase, suggesting regulatory imbalance at the single-cell level. Ongoing work combines fluorescence-based quantification and time-resolved analyses to further resolve the distribution and dynamics of nitrogenase expression under varying redox conditions. This study highlights the importance of single-cell analyses to uncover hidden physiological states in PPB and provides a framework to better understand, optimize, and stabilize hydrogen production in PPB-based processes.

Keywords: Purple phototrophic bacteria; nitrogenase expression; phenotypic heterogeneity; single-cell analysis; fluorescent reporter

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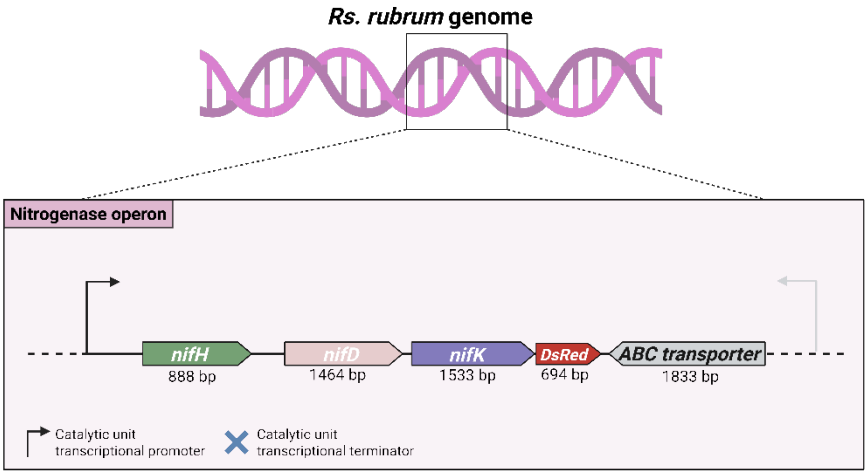


Figure 1. Genetic construction of the *Rhodospirillum rubrum nifK::DsRed* reporter strain. The DsRed coding sequence was inserted at the C-terminus of the chromosomal *nifK* gene using a Cas9-mediated approach, preserving the native transcriptional regulation of the nitrogenase operon (*nifHDK*). DsRed expression is therefore directly coupled to nitrogenase expression. The promoter is indicated by a truncated black arrow and the transcriptional terminator by a pink cross.

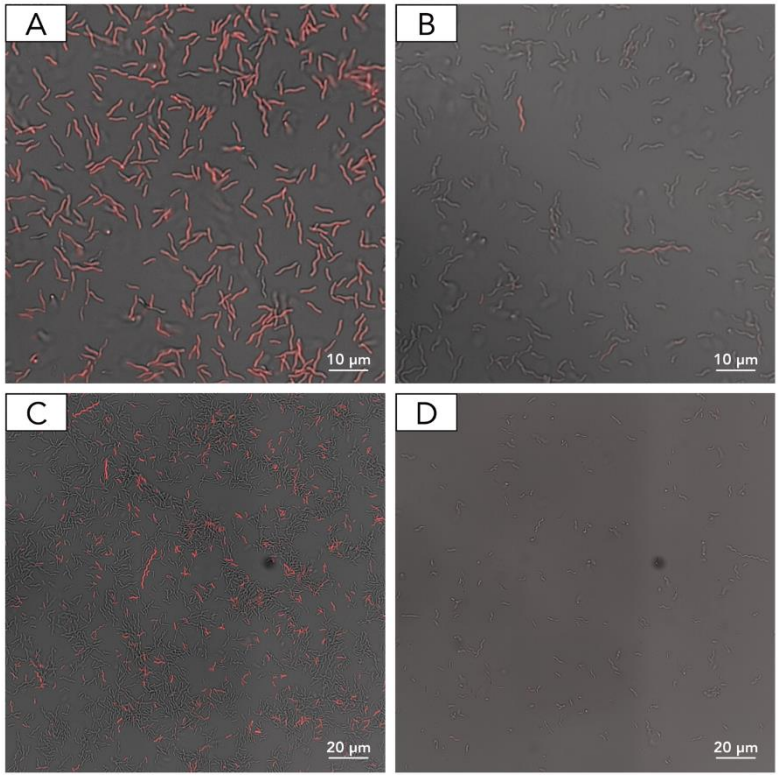


Figure 2. Nitrogenase expression in *Rhodospirillum rubrum nifK::DsRed* at the single-cell level. Epifluorescence microscopy images (overlay of bright-field and DsRed fluorescence) of cells grown under: (A) diazotrophic conditions with a propionate-butyrate mixture, showing homogeneous nitrogenase expression; (B) ammonium-replete conditions with succinate, showing rare fluorescent cells; (C) ammonium-replete conditions with acetate, showing an increased proportion of fluorescent cells under redox and light stress (increased light intensity); and (D) control conditions with no detectable fluorescence. Scale bars: 10 µm (A–B), 20 µm (C–D).

PHYSIOLOGICAL RESPONSE OF PURPLE PHOTOTROPHIC BACTERIA TO THE ELEVATED LEVEL OF MANGANESE IONS

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Metal ions are known to be one of the factors that contribute significantly to environmental pollution. When present in high concentrations, they are toxic to all living species, as they can be incorporated into cells and affect metabolic pathways by altering the production and activity of biomolecules. Purple phototrophic bacteria (PPBs), due to their flexible metabolic modes and relatively high tolerance to exposure to a variety of environmental factors, have gained attention as potential agents in the removal of contaminants from wastewater of different origin. In line with the above, we present the changes in the dynamics of the growth and functionality of PPB induced by the presence of manganese ions. The PPBs were cultured under anaerobic conditions in the presence of a given concentration range of metal ions. Several sigmoidal functions were tested to describe the bacterial growth curves under applied stress factors. Based on the results, the relevant physiological and ecological parameters were obtained. In addition, spectroscopic and biological assays were carried out to specify the effects of the presence of manganese on the morphological changes of the membrane and the light-harvesting properties of PPB. The results point to the potential of PPB in bioremediation of such types of contaminants from the water environment.

Keywords: purple bacteria, environmental pollution, water treatment, growth curve

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MICROENCAPSULATION OF PPB-DERIVED BIOACTIVE COMPOUNDS BY SPRAY DRYING

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Purple phototrophic bacteria (PPB) have gained increasing attention in recent years due to their remarkable metabolic versatility, high protein content, pigment production capacity, and ability to valorize organic waste streams. PPB biomass is rich in high-value bioactive compounds such as single-cell protein (SCP), protein extracts, molecular hydrogen, carotenoids (lycopene and β-carotene), coenzyme Q10, polyhydroxyalkanoates (PHA), 5-aminolevulinic acid (5-ALA), and lipopolysaccharides (LPS). However, many of these compounds, particularly carotenoids, are highly sensitive to heat, light, and oxidation, which can lead to significant losses in their biological activity during processing and storage. Microencapsulation provides a protective physical barrier around bioactive substances, helping to reduce environmental degradation, mask undesirable flavors or odors, and enhance controlled release and bioavailability. Spray-drying microencapsulation has emerged as a cost-effective and industrially scalable approach to improve the stability, storage performance, and practical applicability of these sensitive bioactive compounds in food, nutraceutical, and pharmaceutical applications. Therefore, this review aims to discuss the potential of spray-drying technology for the microencapsulation of PPB-derived bioactive compounds. In PPB-based resource recovery systems, harvesting protein-rich biomass is considered one of the most critical processing steps. Previous studies have shown that spray drying following centrifugation and pasteurization is among the most effective downstream processing strategies, enabling microbial loads to be reduced below regulatory limits while increasing dry matter content above 85%. In addition, spray drying can significantly improve the shelf life of carotenoids by minimizing oxidative degradation. Economic evaluations have also demonstrated that the total harvesting cost, including spray drying, is approximately 400 USD per ton and may be compensated through value-added applications such as fishmeal replacement, organic fertilizers, or functional ingredients. The production of stable PPB powders may also facilitate the incorporation of photoactive biomass into functional food formulations, feed systems, and circular bioeconomy applications. Owing to its rapid dehydration capacity, continuous operation, improved shelf life, and high industrial scalability, spray drying represents a promising post-processing technology for converting PPB biomass into stable, functional, and sustainable bioactive ingredients.

Keywords: Purple phototrophic bacteria, Microencapsulation, Carotenoids

TRHODOPSEUDOMONAS PALUSTRIS RESHAPES RHIZOSPHERE MICROBIOME–METABOLOME NETWORKS: ACHIEVING HIGH LETTUCE YIELD AND NITROGEN USE EFFICIENCY UNDER REDUCED NITROGEN FERTILIZATION IN ACIDIC LATOSOL

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Excessive nitrogen (N) fertilization in acidic latosols reduces nitrogen use efficiency (NUE), aggravates soil acidification, and increases the risk of nitrate loss, threatening sustainable vegetable production. *Rhodopseudomonas palustris* is a free-living N-fixing purple non-sulfur bacterium with potential for reduced-N agriculture; however, how it coordinates rhizosphere microbial, metabolic, and N-cycling processes to sustain crop performance remains unclear. Here, lettuce pot experiments were conducted in acidic latosol under conventional N fertilization, 30% or 50% N reduction, manure substitution, and *R. palustris* inoculation. Plant performance, soil properties, enzyme activities, rhizosphere microbiomes, metabolomes, N-cycle functions, and ^{15}N tracing were integrated with partial least squares path modeling (PLS-PM).

Under 30% N reduction plus *R. palustris* inoculation (0.7CP), aboveground fresh weight and soil pH increased by 26.03% and 5.53%, respectively, relative to conventional fertilization, while soluble protein content increased by 30.18–41.22% and leaf nitrate accumulation decreased. Inoculation also enhanced soil enzyme activities by 9.82-46.00%. *R. palustris* reshaped rhizosphere communities by enriching *Chloroflexi*, *Bacteroidota*, and *Mortierellomycota* and decreasing potentially unfavorable *Chaetothyriales*. Metabolomic profiling revealed upregulation of trehalose, sucrose, isopimaric acid, and liquiritin, associated with stress protection, energy supply, and defense signaling, together with downregulation of sesquiterpene lactone derivatives, including lactucin and lactucopicrin. Consistently, *R. palustris* enhanced N mineralization and microbial immobilization, optimized NH_4^+/NO_3^- partitioning, and promoted carbon fixation, alternative carbon utilization, and N assimilation through the GS/GDH pathways. ^{15}N labeling further showed that N_2 fixation under reduced N reached 7.70-fold that of the control. PLS-PM indicated strong positive effects of *R. palustris* on rhizosphere metabolites (β=0.829), fungal communities (β=0.789), bacterial communities (β=0.739), and soil physicochemical properties (β=0.738).

Collectively, *R. palustris* coordinates soil-microbiome-metabolome-N cycling interactions to improve lettuce productivity, quality, and NUE under reduced N input, providing a promising microbial strategy for sustainable vegetable production in acidic latosols.

Keywords: Rhodopseudomonas palustris; NUE; rhizosphere microbiome; metabolome; 15N tracer;

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TPURPLE FUTURES: QUANTIFYING MARKET ADOPTION BARRIERS FOR PPB COMMERCIALIZATION THROUGH BIOGAS INDUSTRY INSIGHTS

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Purple Phototrophic Bacteria (PPB) offer groundbreaking solutions across bioplastics (PHA), biofertilizers, bioenergy, and wastewater resource recovery. However, their path to commercialization faces complex non-technical barriers strikingly similar to those that have shaped the evolution of the biogas industry. Like biogas, PPB technologies could provide high-value products and renewable energy while addressing environmental externalities, yet both are constrained not by technical feasibility but by market fragmentation, policy misalignment, and stakeholder engagement gaps[1].

To systematically decode these barriers for PPB, we leverage a global data-driven framework validated by biogas industry dynamics. Globally, the biogas industry is struggling despite generous subsidies. Global patterns reveal that subsidy volume alone cannot explain performance disparities; contextual endowments are decisive. We propose that resource endowment and socio‑economic context shape each country's biogas development potential. Using a database that links technical potential (184 countries, 1960–2022) with realized production (99 countries, 1990–2021) , we measure industry maturity as the ratio of output to potential.

Applying interpretable machine learning (XGBoost and Random Forest with SHAP) to this database, we identify three dominant drivers: environmental taxation, electricity prices and organic waste availability. Residential electricity tariffs above 180 USD/ MWh sharply improve returns, and renewable energy shares below 30% correlate with higher biogas returns, but beyond this threshold, sector competitiveness declines due to subsidy competition with other renewables. Our framework groups countries into high, medium‑ and low‑return clusters, enabling governments to direct support where it yields the greatest social benefit.

These biogas-validated insights directly inform PPB commercialization strategy. For instance, PPB-based biofertilizers require co-developed supply chains with farmers; biohydrogen projects need carbon credit mechanisms to offset costs; wastewater applications demand regulatory frameworks for nutrient trading. By clustering regions based on high/medium/low-return clusters, our framework enables targeted deployment—prioritizing contexts where PPB can maximize societal returns per public investment. This data-driven approach bridges the gap between laboratory innovation and market-ready bioeconomy solutions, accelerating the purple revolution.*Keywords: Purple Phototrophic Bacteria, Transcriptomics, Bio-electrochemical Systems, Resource Recovery, mRNA-seq*

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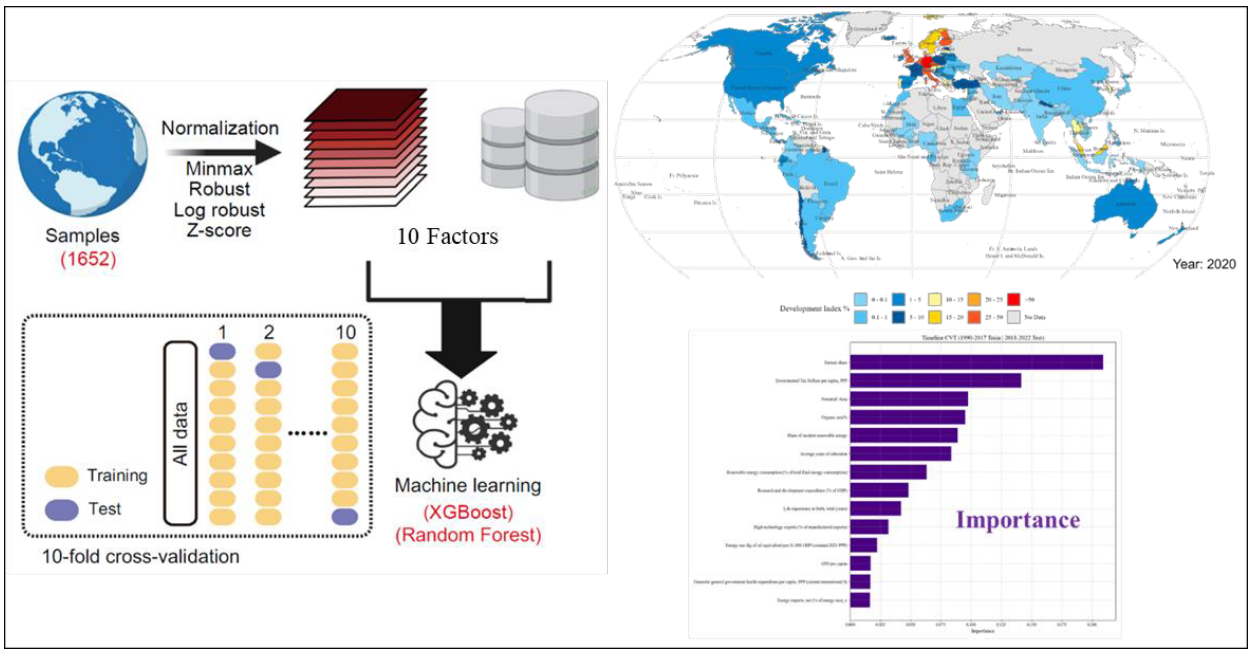


Figure 1. Global mapping of global biogas industry development and key factor importance.

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RHODOPSEUDOMONAS PALUSTRIS ENHANCES NITROGEN UTILIZATION EFFICIENCY AND LETTUCE PRODUCTIVITY UNDER REDUCED FERTILIZATION BY ORCHESTRATING RHIZOSPHERE MICROBIOME–METABOLOME NETWORKS IN ACIDIC LATOSOL

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Excessive nitrogen fertilization in acidic latosols frequently compromises nitrogen use efficiency (NUE), exacerbates soil acidification, and disrupts rhizosphere ecological functions. Although *Rhodopseudomonas palustris* are known to fix nitrogen, how they orchestrate rhizosphere microbiome–metabolome interactions to sustain crop productivity under reduced nitrogen input remains largely unclear. This study conducted pot experiments in acidic latosol, under conventional nitrogen fertilizer, reduced nitrogen fertilizer input, manure substitution, and *R. palustris* inoculation. And partial least squares path model (PLS-PM) was used for analysis. *R. palustris* effectively alleviated the adverse effects of nitrogen reduction and maintained or improved lettuce productivity and quality. Under reduced nitrogen input, *R. palustris* inoculation increased soluble protein content by 30.18-41.22%, reduced nitrate accumulation in leaves, and enhanced soil enzyme activities by 9.82-46.00%. Among all treatments, reducing nitrogen fertilizer by 30%, and adding *R. palustris* (0.7CP), then use manure instead of chemical fertilizer on this basis (0.7CMP), improved crop yield and quality, effectively enhanced soil physicochemical properties and enzyme activity. Compared with conventional fertilization, Fresh weight of crop aboveground part increased by 26.03% and 30.22% in 0.7CP and 0.7CMP, respectively, while soil pH increased by 5.53% and 9.20% in 0.7CP and 0.7CMP, respectively. Rhizosphere analysis showed that *R. palustris* reshaped microbial communities by enriching beneficial groups, including Chloroflexi, Bacteroidota, and Mortierellomycota, while reducing potentially unfavorable Chaetothyriales. Metabolomic analysis revealed that rhizosphere metabolites such as Trehalose, Sucrose, Isopimaric acid, and Liquiritin, which are involved in stress protection, nitrogen fixation energy supply, defense, and signaling in nitrogen-fixing bacteria, were significantly upregulated in the 0.7CP and 0.7CMP treatment groups. Meanwhile, metabolites such as Sesquiterpene lactone derivative, Lactucin, and Lactucopicrin, which may inhibit nitrogen-fixing bacterial activity and compete for energy resources from nitrogen-fixing bacteria, were significantly downregulated. PLS-PM showed strong positive effects of *R. palustris* on rhizosphere metabolites ($\beta = 0.829$), fungal communities ($\beta = 0.789$), bacterial communities ($\beta = 0.739$), and soil physicochemical properties ($\beta = 0.738$). Collectively, *R. palustris* functions as a rhizospher activity orchestrator of soil-microbiome-metabolome networks, enabling nitrogen efficient and sustainable lettuce production in acidic latosols.

Keywords: Rhodopseudomonas palustris; reduced nitrogen input; lettuce; nitrogen use efficiency; rhizosphere microbiome; metabolome; acidic latosol

FOOD-GRADE SUPRAS SYSTEM FOR SUSTAINABLE RECOVERY OF PIGMENTS FROM RHODOPSEUDOMONAS PALUSTRIS

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Purple phototrophic bacteria (PPB), such as *Rhodopseudomonas palustris*, represent a promising and underexploited source of natural pigments, including carotenoids and bacteriochlorophylls, with potential applications in the food industry. Conventional pigment extraction methods, however, typically rely on toxic organic solvents, limiting their use in food-related products. Supramolecular solvents (SUPRAS) offer a green, food-compatible alternative combining high extraction efficiency with reduced environmental impact. Several SUPRAS formulations based on octanoic acid and water, combined with ethyl acetate, ethanol or acetone as a third component, were screened and characterized in terms of phase volume, water content and density. The system composed of octanoic acid, ethyl acetate and water was selected as the most suitable, showing the lowest water content and the most reliable phase separation, together with compatibility with food-sector applications. This system was applied for pigment extraction from *R. palustris* biomass following an experimental design in which sample mass, extraction temperature and extraction time were varied, and extracts were characterized spectrophotometrically for total carotenoid and chlorophyll content, before and after saponification, as well as for antioxidant activity by the ABTS assay. Total carotenoid content of the SUPRAS extracts reached up to 24.0 mg/100 g before saponification and 26.7 mg/100 g after saponification, considerably higher than that obtained by conventional organic-solvent extraction (1.3–5.1 mg/100 g), confirming the superior extraction capacity of the developed system. The highest total chlorophyll content (14.0 mg/g) was achieved at 23 °C with a 120-min extraction time, while the strongest antioxidant activity (up to 112 mM TEAC/100 g) was recorded in extracts obtained at 80 °C, exceeding the antioxidant activity of the organic-solvent reference. These results indicate that extraction efficiency depends on the interaction of processing parameters rather than any single factor. The developed food-grade SUPRAS system demonstrates strong potential as a sustainable, non-toxic alternative to conventional solvents for the efficient recovery of bioactive pigments from PPB biomass, supporting the valorisation of microbial biomass in food-related applications.

Keywords: SUPRAS, pigment recovery, food application, purple phototrophic bacteria, antioxidant activity

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ULTRASOUND-ASSISTED EXTRACTION AS A SUSTAINABLE TOOL FOR *SPIRULINA PLATENSIS* PIGMENTS RECOVERY

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Due to its nutritional properties, *Spirulina* represents one of the most commonly used microalgae nowadays. Regarding its positive effects, *Spirulina* has been certified as Generally Recognized as Safe (GRAS) by several countries. The common name *Spirulina* (SP) refers to an ancient lower prokaryote belonging to Cyanophyta, Cyanophycean, and Oscillatoria, also known as *Spirulina platensis* (*S. platensis*) or *Arthrospira platensis* (*A. platensis*). This microscopic cyanobacterium can be found in tropical and subtropical alkaline, high-salinity waters. Microalgae have gained significant attention as remarkable sources of biologically active compounds with different useful properties owing to the presence of diverse chemicals, including proteins, minerals, carbohydrates, and phytopigments. Additional bioactive components contributing to the color of these algae include chlorophyll, carotenoids, and phycocyanin. In order to isolate the most prominent pigments in *Spirulina platensis*, a novel and green Ultrasound-assisted technique (UAE) was applied. As a solvent, 70% w/w ethanol was used, and as variables, extraction time (5 min, 10 min, 15 min) and UAE amplitude (30%, 50%, 70%) were examined. According to the HPLC results, the highest content of carotenoids, specifically lycopene (0.55µg/ml) and astaxanthin (10.51µg/ml), was detected in the sample obtained under conditions of 5 minutes and 30% amplitude, and 15 minutes and 70% amplitude, respectively, while the highest content of Chlorophyll a was detected in the sample obtained under conditions 10 minutes and 30% amplitude. Based on these results, the future of applying *Spirulina* for obtaining pigments for the food, pharmaceutical, or cosmetics industry is promising, especially while isolating bioactive compounds by using environmentally friendly extraction techniques such as UAE.

Keywords: Spirulina platensis, Ultrasound-assisted extraction, carotenoids, chlorophylls

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INNOVATIVE NADES-BASED APPROACH FOR *SPIRULINA PLATENSIS* PIGMENTS RECOVERY

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The use of natural products for isolating valuable bioactive compounds has expanded in recent years. In the literature, microalgae are presented as one of the dominant natural sources, especially for pigments such as carotenoids and chlorophylls. Based on these facts, *Spirulina platensis* was used in the present study to optimize the isolation of carotenoids and chlorophylls. In terms of targeting the environmental and economic goals simultaneously, the isolation of pigments was performed using a novel and green Ultrasound-assisted extraction (UAE) technique. A step forward in reducing the negative footprint of the extraction technologies on the environment was achieved by using Natural Deep Eutectic Solvents (NADES). Therefore, NADES optimisation was performed in this study. As a variable, a solid/liquid ratio (S/L) of 1/10, 1/20, and 1/30 was used. Additionally, the UAE amplitude was varied from 20-60%. The impact of extraction conditions, while using Glycerol-Betaine (3:1) as a solvent, was examined. Based on the HPLC results, the most prominent extract in terms of carotenoid isolation, specifically astaxanthin (35.83 μg/mL), was the extract obtained after 10 min of extraction at an amplitude of 20%, and an S/L ratio of 1/10. On the other hand, the highest content of chlorophyll a (7.90 μg/mL) was determined in the extract obtained after 10 min of extraction at an amplitude of 60%, and an S/L ratio of 1/10. Based on the presence of β-carotene and also lycopene, the approach of using UAE integrated with NADES shows a promising perspective in innovative isolation of pigments, with potential for a broad range of applications, including application in pharmaceutical, cosmetic, or food industries.

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Keywords: Spirulina platensis, NADES, Ultrasound-assisted extraction, carotenoids, chlorophylls

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BIOCONVERSION OF CHEESE WHEY USING PURPLE PHOTOTROPHIC BACTERIA: METABOLIC STRATEGIES, PROCESS CHALLENGES, AND FUNCTIONAL APPLICATIONS

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Cheese whey, one of the major by-products of the dairy industry, poses significant environmental challenges due to its high organic load, while simultaneously representing a valuable biotechnological resource owing to its rich composition in lactose, proteins, and minerals. Traditionally treated as waste, whey is now increasingly recognized as a sustainable feedstock for biomass generation and value-added product recovery. In this context, purple phototrophic bacteria (PPB) have emerged as promising microbial platforms due to their versatile photoheterotrophic metabolism, enabling the conversion of organic-rich waste streams into high-value compounds under anaerobic, light-driven conditions. The bioconversion of whey using PPB systems has gained growing attention, particularly in relation to metabolic pathway optimization and integration into food and feed applications. However, several intrinsic limitations hinder process efficiency, including the inability of most PPB strains to directly metabolize lactose, reduced light penetration caused by medium turbidity, and the impact of protein content on rheological properties and mass transfer. To overcome these constraints, pre-treatment strategies such as enzymatic lactose hydrolysis, acidogenic fermentation, and membrane filtration have been proposed. In addition, co-culture systems incorporating lactic acid bacteria have proven effective in converting lactose into readily assimilable substrates (e.g., organic acids), thereby enhancing substrate utilization, biomass yield, and metabolic diversity. Various photobioreactor configurations, including tubular, flat-panel, and biofilm-based systems, have been explored for whey-based PPB cultivation. Nonetheless, challenges related to light distribution, oxygen sensitivity, and biomass retention remain critical for scale-up. PPB-derived biomass is characterized by high protein content and a favorable essential amino acid profile, alongside the production of functional compounds such as natural pigments, antioxidants, and bioactive lipids. Furthermore, these systems offer potential for symbiotic applications by enhancing probiotic stability and modulating gut microbiota. Notably, certain PPB-derived metabolites have demonstrated antimicrobial activity against foodborne pathogens, including *Listeria monocytogenes* and *Salmonella* spp. Future perspectives include the development of lactose-utilizing PPB strains, optimization of hybrid microbial consortia, implementation of AI-driven process control strategies, and the integration of whey valorization into dairy-based biorefinery concepts. Overall, PPB-based conversion of whey represents a sustainable and innovative approach to transforming dairy waste into high-value bioproducts, contributing to circular bioeconomy goals and improved environmental performance of the dairy sector.

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A LIFE CYCLE ASSESSMENT FRAMEWORK FOR QUANTIFYING ENVIRONMENTAL IMPACT IN PPB SYSTEMS

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Purple phototrophic bacteria (PPB) systems are advancing as solutions for wastewater treatment and the recovery of biomass, nutrients and other bioproducts. However, their environmental assessment is constrained by challenges common to emerging technologies, including sparse and scale-dependent foreground data, uncertain process configurations and the limited availability of PPB-specific unit processes in conventional life cycle inventory (LCI) databases (i.e. Ecoinvent). Consequently, existing assessments rely on study-specific combinations of proxy datasets, literature-derived inventories, engineering calculations and allocation or substitution assumptions, limiting their transparency, comparability and transferability. We developed a methodological and guidance framework to support PPB researchers and technology developers in constructing transparent, reproducible LCA inventories and methods for PPB-based technologies, advancing current practice beyond study-specific, undocumented workarounds. The framework comprises two complementary components. First, a decomposition-based approach disaggregates the unavailable PPB process into components with database analogues (energy demand, infrastructure or material proxies, key inputs) and estimates the rest from mass or elemental balances, following precedent from microalgae-cultivation LCA studies. Second, the framework provides a structured hierarchy for selecting data sources according to their intended function: Ecoinvent for generic inputs, Agri-footprint or the World Food LCA Database when benchmarking PPB protein or biomass against conventional feed and protein sources, and literature-derived inventories, clearly labelled as such, for the PPB process itself. The framework is demonstrated through an illustrative case study comparing a PPB-retrofitted wastewater system with a conventional baseline, using illustrative, non-measured data on climate-change and eutrophication impacts. The two impact categories pull in different directions — climate performance improves, eutrophication gets worse — to make a point that's easy to state but easy to miss in practice: carbon-only reporting can misrepresent how a PPB system performs, and how data gaps and allocation choices are handled changes which trade-offs are visible and must be highlighted and communicated during the interpretation stage. We present this framework as a practical starting point for PPB researchers and technology developers seeking to assess PPB systems despite current methodological and data limitations, improving the transparency, reproducibility and comparability of LCA studies in this field. Although developed for PPB systems, the framework offers a structured approach adaptable to environmental assessment of other emerging biotechnologies facing similar challenges.

Keywords: Life cycle assessment, purple phototrophic bacteria, data gaps, eutrophication, wastewater treatment

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LIFE CYCLE ASSESSMENT AND TECHNO-ECONOMIC ANALYSIS OF INDUSTRIAL WASTEWATER

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The treatment of complex and recalcitrant industrial effluents poses a significant environmental and economic challenge. Solar-driven photocatalytic reactor systems have emerged as a promising advanced oxidation process (AOP) for the degradation of persistent organic pollutants, offering a potential pathway for sustainable wastewater management. Also, investigated the environmental performance and techno- economical viability of producing solid biofuel and biofertilizer from microalgae. While the technical efficacy of these systems has been demonstrated at a laboratory scale, their real-world viability hinges on both environmental and economic performance. This study conducts a comprehensive assessment of solar photocatalytic reactor systems for industrial wastewater treatment by integrating a Life Cycle Assessment and a Techno-Economic Analysis. The evaluates the environmental footprint across the entire life cycle of the system, from raw material acquisition and manufacturing to operation and end-of-life disposal, quantifying impacts such as global warming potential, energy consumption, and resource depletion. Concurrently, the provides a detailed economic evaluation, estimating capital expenditures, operational expenditures, and the levelized cost of treatment to determine economic feasibility and identify key cost drivers. The results reveal a critical trade-off: while utilizing sunlight as a free energy source reduces operational costs and environmental impacts during the use phase, the embodied energy and cost of photocatalyst synthesis and reactor fabrication are significant. This integrated approach provides a holistic framework for identifying optimization strategies to enhance both the sustainability and economic competitiveness of solar photocatalytic technologies, thereby guiding their development for large-scale industrial application.

Keywords: *life, industrial, wastewater, techno-economic*

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FROM WASTE TO VALUE: VALORISING CHEESE WHEY THROUGH PURPLE PHOTOTROPHIC BACTERIA FOR SINGLE - CELL PROTEIN PRODUCTION

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Dairy products represent a key component of the human diet due to their high nutritional value. During cheese production, only about 10–20% of the processed milk is converted into cheese, while the remaining 80–90% results in whey as a by-product. Over 200 million tons are produced worldwide annually, with the European Union contributing around 40 million tons. Among different feedstocks, cheese whey is the most relevant by-product of the dairy industry, rich in lactose, proteins, and mineral salts. Approximately 50 % of cheese whey is used for high value compounds like whey protein concentrates, and lactose derivates, or for animal feeding, while the rest must be treated before being discharged into the environment. This is due to its high chemical oxygen demand and biochemical oxygen demand up to 102 and 60 gL^{-1}, respectively which should be properly managed. Single-cell proteins, on the other hand, have emerged as a promising solution for sustainable and nutritious protein sources, with a lower water footprint and land requirement, suitable for use in both food and feed.

The aim of this paper is to explore cheese whey upcycling pathways through purple phototrophic bacteria for single - cell protein production, as one application of different waste and by-product streams as economically viable substrates for purple phototrophic bacteria biomass growth. Derived from the biomass of microorganisms such as bacteria, yeast, fungi, and algae, single-cell proteins offer a high-value protein alternative that can be produced with high cost-effectiveness. Among various microorganisms, the use of purple phototrophic bacteria represents a promising approach for single-cell proteins production, which can serve as a substitute or supplement to feed components such as soybeans and fishmeal, which are often subject to price volatility and environmental concerns. The high protein content of purple phototrophic bacteria biomass, up to 80 % on dry matter basis, makes it an excellent source of essential amino acids necessary for animal performance and health. Additionally, purple phototrophic bacteria biomass holds potential as a sustainable alternative to conventional plant protein sources used in feed formulations, such as soybeans. To this end, the valorization of deproteinated cheese whey via purple phototrophic bacteria cultivation could be a good choice for environmental concerns and circular economy.

Keywords: Circular Economy, Whey Valorisation, Single Cell Protein, Purple Phototrophic Bacteria

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ISOBUTANOL PRODUCTION BY GENETICALLY-ENGINEERED *CUPRIAVIDUS NECATOR*

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The strong dependence on non-renewable fuels (oil, coal, natural gas) represents a critical global domain that requires attention due to greenhouse gas emissions, high energy consumption and natural resources depletion; representing a global threat to future energy security, public health, and environmental stability. In this context, biofuels, such as isobutanol, represent highly attractive renewable alternatives due to their high heating power and low hygroscopicity. This work is focused on the production of a sustainable solar biofuel through an artificial photosynthesis system based on the synergistic integration of renewable energy sources and microbial conversion pathways.

The autotrophic metabolism of *Cupriavidus necator* (*C. necator*) represents a potential alternative for biofuels synthesis, in which hydrogen (H_2) can be used as energy source and carbon dioxide (CO_2) as carbon source. However, genetic engineering interventions are required to optimize the metabolic routes of the bacteria and enhance the production of the biofuel. Moreover, the development of growth protocols in which the optimal parameters are stablished are necessary to ensure an efficient use of gases (CO_2 and H_2).

Herein, the aim of this study is the determination, optimization and assessment of the autotrophic growth conditions of different genetically-engineered *C. necator* strains for the enhancement of isobutanol production at lab-scale. Furthermore, substrate supplementation strategies were evaluated through the addition of metabolic intermediates (fructose, pyruvate, and 2-ketoisovalerate) to improve isobutanol production.

Keywords: autotrophic-growth, biofuel, metabolic routes

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EFFECTS OF RESIDUAL IONIC LIQUIDS IN SALT-RICH AQUEOUS PHASES FROM BIPHASIC WASTEWATER TREATMENT SYSTEMS ON GREEN MICROALGAE UNDER MIXOTROPHIC CONDITIONS

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Ionic liquids (ILs) are increasingly regarded as potentially greener extraction media in separation and purification processes because of their low volatility, tunable physicochemical properties and potential recyclability. IL-based aqueous biphasic systems (ABS) have attracted considerable attention for wastewater treatment due to their efficient extraction of contaminants. However, incomplete IL recovery may leave residual ILs in the salt-rich aqueous phase after biphasic separation, creating a potential pathway for their release into aquatic environments and raising concerns about their effects on photosynthetic microorganisms. This study evaluates how IL cation chemistry influences the physiological responses of green microalgae exposed to residual salicylate-based ILs in post-separation salt-rich aqueous phases. To isolate cation-specific effects, green microalgae are exposed to BG-11-adjusted salt-rich aqueous phases containing residual salicylate-based ILs that differ in cation structure. The 72 h exposure is performed in a multicultivator system under mixotrophic conditions, with citrate serving as the organic carbon source. Algal growth and physiological status are assessed using optical density at 680 and 720 nm, dry weight, pigment content, pH and photosynthetic performance parameters, including the maximum and effective quantum yields of photosystem II (Fv/Fm and Φ_{PSII}, respectively). In addition, FTIR profiling is used to explore potential changes in the biochemical composition of biomass and organic signatures associated with residual ILs. IL concentrations and dissolved organic carbon (DOC) are quantified in the supernatant before and after exposure to characterize changes in the exposure medium and provide insight into citrate-associated organic carbon consumption under mixotrophic growth conditions. Overall, this work provides insight into cation-specific effects of residual ILs on microalgae and supports the preliminary ecotoxicological evaluation of post-separation salt-rich aqueous phases from IL-based ABS used for wastewater treatment.

Keywords: Ionic liquids, microalgae, mixotrophy, photosynthetic performance

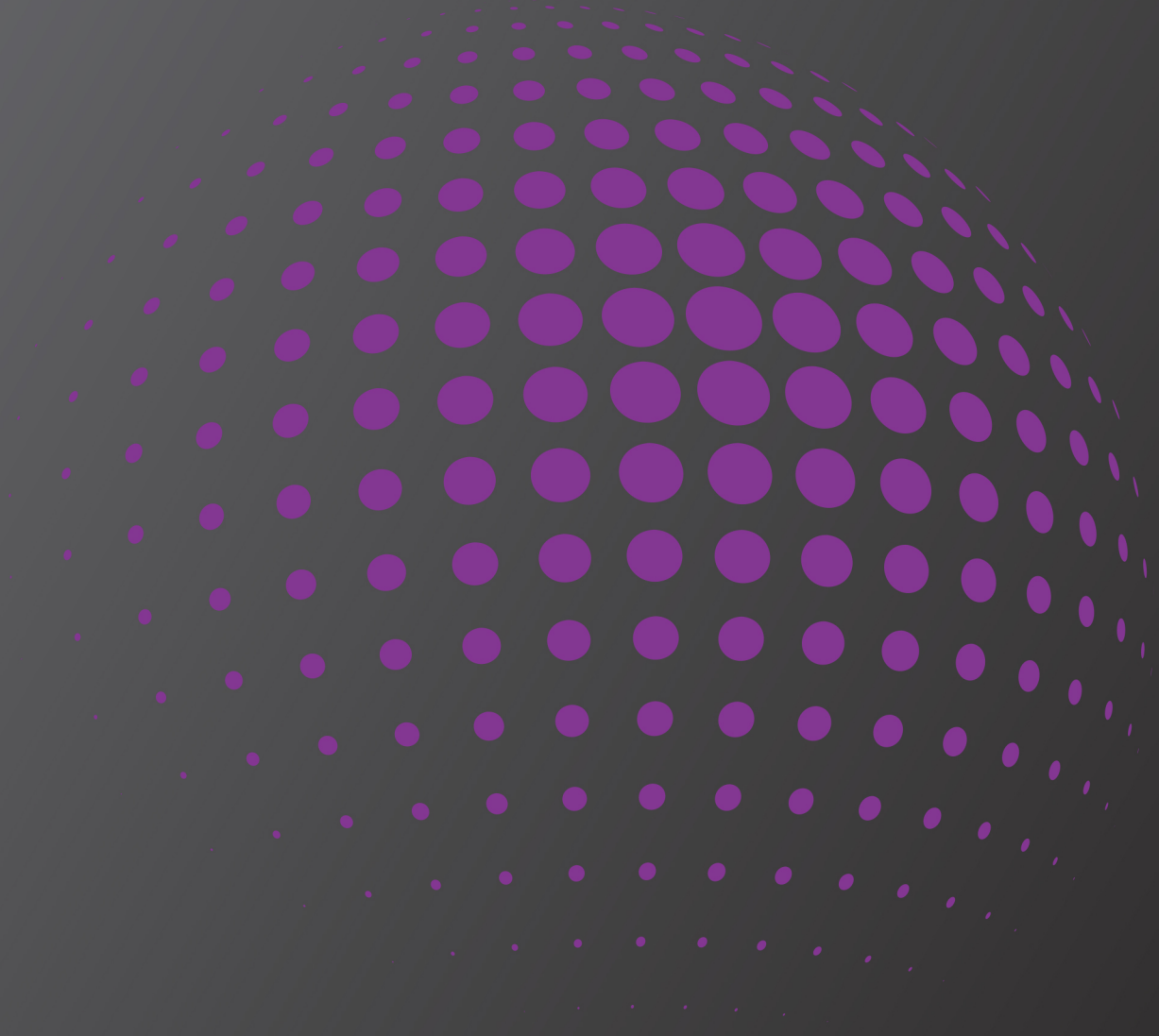
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