Doctoral Program in Chemical Engineering (36th Cycle)

PhD candidate:
Giulia Usai

Supervisors:
Prof. Debora Fino
Dr. Barbara Menin
Dr. Angela Re

Green Chemicals via Photosynthetic Microbial Factories: Integrating Metabolic and Bioprocess Engineering for 2-Phenylethanol Production and CO2 Valorization

ABSTRACT

Designing and optimizing photosynthetic microbial factories for chemicals bioproduction and CO_2 utilization is a complex, multidisciplinary endeavor merging genetics, microbiology, biochemistry, and engineering. This process includes selecting suitable photosynthetic microorganisms (e.g., cyanobacteria or microalgae), investigating metabolic pathways, enhancing carbon fixation, optimizing culture conditions (e.g., light, CO_2 supply, temperature), scaling up production, and continually refining the process to meet environmental and economic sustainability goals. The prokaryotic cyanobacteria, due to their high photosynthetic CO_2 conversion rates, minimal nutritional requirements, and genetic and bioinformatic tools, stand out as ideal candidates for these tasks.

Particularly, this thesis focused on developing a photosynthetic microbial factory combining metabolic and bioprocess engineering to produce 2-phenylethanol (2-PE) and valorize CO_2. 2-PE is a rose-scented aromatic compound and finds applications in cosmetics, pharmaceuticals, food and beverages. While plants naturally synthesize 2-PE via shikimate pathway (SKP), its extraction is costly and low-yielding, driving most production to chemical synthesis using petroleum feedstocks, and resulting in unwanted by-products and health issue. A previously published engineered strain of *Synechococcus elongatus* PCC 7942, named p120, heterologously expressed four genes to synthesize 2-PE. In the present research a combination of metabolite doping and metabolic engineering was employed to enhance 2-PE production profile in p120, resulting in a new strain, i.e. '2PE_*aroK*'. Thus, by overexpressing the native shikimate kinase (*aroK*) and subjecting it to L-phenylalanine doping, the 2-PE production significantly increased up to 285 mg L^{-1}, 2.4-fold higher than p120, and ultimately the highest reported via photoautotrophy in the literature.

To further enhance the 2-PE production, the 2PE_*aroK* mutant strain was assessed in a lab-scale flat panel photobioreactor (PBR) under several light conditions, ranging from darkness to varying photon flux levels (100-500 μmol photons m^{-2} s^{-1}). Notably, in the darkness neither biomass accumulation nor

2-PE production were observed, stressing the pivotal role of the light in driving the bioprocess. The results highlighted 150 μmol photons m^{-2} s^{-1} as the optimal setting, yielding 282 mg L^{-1} 2-PE and a productivity of 28.7 mg L^{-1} d^{-1}, which represents one of the most significant goals of this study. Conversely, the highest light condition decreased the 2-PE production by 30%. Additionally, carbon balance analysis showed that at lower light intensities, 45-50% of carbon atoms partitioned into 2-PE synthesis, while at higher light, it decreased to 28%, with the remaining carbons directed towards biomass formation. Therefore, increasing the carbon flow through the photosynthesis did not expand inevitably the target production, while the excess of carbon and energy is preferentially regulated via biomass accumulation.

Once the metabolic link between photosynthesis and 2-PE kinetics got established in 2PE_*aroK*, the study progressed to scale up the bioprocess. Four geometrically different PBRs were assessed for biomass and 2-PE productivities. Although the highest biomass productivities of the thesis were reached in this pool of tests, severe biological contaminations hindered the bioprocess, ultimately failing in 2-PE synthesis and crashing the entire cultivation. Only the stirred tank yielded 2-PE, albeit at half the titer achieved in smaller scale tests, even if no contamination appeared. This study highlighted the need for further studies to identify the optimal environmental and operative conditions in larger scale bioprocess design.

To address sustainability, the 2PE_*aroK* strain was cultivated in dairy wastewaters. Four dairy wastewaters were screened and, the liquid effluent of exhausted sludges (ES) and washing waters from milk tanks (WW) proved suitable for cyanobacterial cultivation. Thus, these waste streams were combined in several ratios and the mixture of 75% ES and 25% WW resulted in 180 mg L^{-1} 2-PE production and a productivity of 18.03 mg L^{-1} d^{-1}, overall enhancing the bioprocess sustainability.

To sum up, this thesis outlines a comprehensive approach to develop a photosynthesis-based bioprocess that has successfully achieved the production of high-value aromatic compound and CO_2 utilization. The research effectively integrated metabolic and bioprocess engineering while highlighting the strengths, applications, and challenges that bioprocess engineering faces when addressing photosynthetic microbial factory for sustainable production of green chemicals and CO_2 valorization.