Doctoral Dissertation
Doctoral Program in Chemical Engineering (37th Cycle)

PhD Thesis Summary – Giacomo Proietti Tocca

Towards sustainable food production: studies on CO_2, acetic acid and formic acid valorisation into microalgal biomass rich in proteins and oils with high nutritional value

Sustainable technologies are critical for European economic growth and achieving European Commission's Green Deal's targets of climate neutrality by 2050, which require a 90% reduction in greenhouse gas (GHG) emissions by 2040. With the global population projected to reach 9.8 billion by 2050, agriculture must increase food production while minimizing its environmental impact. Currently, agriculture contributes 26% of global GHG emissions, half of which stem from livestock farming, which also uses 40% of cropland, exacerbating pollution, biodiversity loss, and eutrophication. A "protein transition" toward alternative, sustainable protein sources is consequently mandatory. Alternative proteins are emerging as a solution, replicating the taste, texture, and nutrition value of animal products while addressing environmental and ethical concerns. These include plant-based proteins, microbial biomass, and cell-based cultivated meat. Among these, microalgae stand out due to their high protein content (up to 65% dry weight), well-balanced essential amino acid profile, and ability to produce valuable compounds like fatty acids and pigments. Microalgae are a sustainable nutritional source for animals (especially fish) and humans.

Conventional microalgae production faces significant challenges. Phototrophic cultivation is limited by sunlight availability, self-shading, and the need for high-cost photobioreactors, while inefficient CO_2 mass transfer further constrains yields. Fermentation-based production through heterotrophic cultivation also suffers from high costs and environmental impacts associated with sugar substrates. Mixotrophy, which combines sugars with light as an energy source, shares these limitations.

This work explores acetic acid (AA) and formic acid (FA) as alternative carbon sources for microalgal heterotrophic and mixotrophic growth. The main aim is to propose novel carbon feedstocks that can be produced through innovative processes starting from CO_2, to increase both economic and ecological sustainability of microalgal biomass production without compromising its nutritional quality. Both AA and FA can be derived from CO_2 through innovative processes like gas fermentation, electrocatalysis and microbial electrosynthesis, potentially improving the economic and ecological sustainability of microalgae production. Two different studies are here proposed:

The first study presents an innovative two-step process valorising acetate produced through *Thermoanaerobacter kivui* gas fermentation as carbon source for *Chlorella sorokiniana* heterotrophic cultivation. The real acetate-rich stream (13.3 g L^{-1}) was tested at different dilutions, achieving biomass productivities up to 1.61 g L^{-1} day^{-1}. The heterotrophic biomass had high protein content (≈ 45-50% dry weight) and a balanced essential amino acid profile suitable for human and fish nutrition. Lipids contained valuable omega-3 fatty acids (ω6:ω3 ≈ 1). The nutritional potential of *T. kivui* biomass collected from the centrifugation of the gas-fermentation stream was evaluated, obtaining its amino acids profile and nitrogen to protein conversion factor (2.89). This innovative process, combining gas fermentation with microalgae cultivation, offers a promising approach to enhancing proteins and lipids production for food and feed applications, compared to traditional photoautotrophic methods.

In the second study, FA and AA were tested as carbon sources for the mixotrophic growth of the acidophilic strain *Galdieria sulphuraria*. Tests on microplates identified very low inhibitory thresholds concentrations (0.100 g L^{-1} for FA and 0.300 g L^{-1} for AA), making batch cultivation unfeasible. However, in two-liter flat-panel reactors, a fed-batch strategy allowed the effective use of FA and AA, achieving high biomass yields (90–100% $gC_X gC_S^{-1}$), outperforming the limited yields of autotrophic CO_2-based cultivation (< 20%). FA was metabolized via NAD-dependent formate dehydrogenase (FDH), making *G. sulphuraria* the first wild-type microalgal strain known to metabolize FA. The photosynthetic activity increase the metabolic energy and allow to obtain very high yields, which were not observed in other native (and non-photosynthetic) formatotrophic microorganisms. However, when compared to conventional sugars, FA proved inefficient as energy source: biomass productivity showed no significant improvement compared to photoautotrophic conditions. AA, which participates in cellular respiration, delivered more energy and boosted autotrophic biomass productivity by 19%. However, this increase was still lower compared to the twofold productivity observed in neutrophilic strains. Analysis of the amino acid composition and nitrogen content verified that the biomass quality remained consistent irrespective of the carbon source or feeding rate. Due to its high yield and the potential for sustainable production via electrocatalysis, FA emerges from this study as a promising alternative to CO_2 as a carbon source for *G. sulphuraria*.