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Doctoral Program in Materiali, processi sostenibili e sistemi per la transizione
energetica (38th Cycle)

Microalgae-based biodiesel: strategies to develop a sustainable biochemical process

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Abstract

After industrialization, human activities began to increase Green House Gasses (GHG) emissions with a clear effect on environmental and human health. The continuously increasing population and energy demand dramatically enhanced global warming. Right now, more than ever, we must face this urgent crisis by reducing emissions and replacing fossil-based energy with renewable alternatives. In this context, transport sector is responsible for majority of oil consumption and just 11.2% of energy used in this sector is renewable, far from the 29% target set by 2030. To achieve this goal new technologies are required.

Liquid biofuels mainly derive from crops, agricultural residues or cooking oils, but they are not enough to meet the growing demand for biofuels. Microalgae represent an alternative feedstock for biodiesel production. They are 20 times more productive than crops and do not require arable land utilization. However, nowadays microalgae-based biodiesel is not yet economically feasible. For these reasons, more efforts are needed to improve the production of this valuable biomass.

In this thesis three cultivation strategies to improve biomass and lipid production in microalgae have been employed. Overall, microalgae have been cultivated on industrial wastewater to reduce costs and increase sustainability. The utilization of streams from dairy industry resulted to be a valid solution for the cultivation of both *Chlorella sorokiniana* and *Tetrademus obliquus*. When *C. sorokiniana* was cultivated in a balanced substrate obtained with 75% of dairy washing waters (DWW) and 25% of dairy exhausted sludge (DES) high biomass productivity ($0.052 \text{ gDW L}^{-1} \text{ d}^{-1}$) was achieved.

This substrate was used for the cultivation of *C. sorokiniana* in a multicultivator PBR under different light regimes to improve lipid productivity and quality. A two-step cultivation process was designed, consisting of initial biomass accumulation followed by a shift in light wavelength to evaluate its effect on lipid fraction. Red light (660 nm) achieved the highest biomass productivity ($0.102 \text{ gDW L}^{-1} \text{ d}^{-1}$) and lipid content (40.75% of dried biomass). In contrast, blue light drove to an increase in biodiesel quality in terms of oxidative stability and ignition quality but showed poor low-temperature performances (high CFPP).

Notably, when red light was implemented in the first step followed by blue light (R100-B300), CFPP was halved.

Nitrogen limitation is known to boost lipid productivity in certain microalgae, thus a two-step process involving different species in each stage was conducted. Mixed dairy effluent (MDE) from an Irish industry was used as substrate. The highest lipid content both in first and second steps were obtained when *T. obliquus* was cultivated in the first stage followed by *Nannochloropsis oceanica* (MDE-TET-NO) with 27.14% and 43.89% of lipid, respectively. Low oxidative stability was observed in the first step and low ignition performance in the second, thus by blending two biomasses higher quality of biodiesel is achieved. Coupling species with distinct lipid profiles is a promising strategy to improve the quality of final product.

Finally, *C. sorokiniana* was cultivated on a carbon-rich substrate derived from a valuable gas fermentation process. Here high biomass productivity ($0.295 \text{ gDW L}^{-1} \text{ d}^{-1}$) was obtained, together with high-quality biodiesel. High biomass productivity confirmed the feasible contribution of microalgae in valorising GFE. The cultivation of valuable biomass on GFE strongly improved the sustainability of the gas fermentation process, toward a circular process with zero-waste and high-value products.

Overall, biodiesel production was coupled with wastewater treatment. Nitrogen removal reached 50%, 99% and 100%, while phosphorus removal is 35%, 87% and 100% respectively for R100-B300, MDE-TET-NO and 50:50 DES:GFE cultivations. These results highlight the sustainability of these strategies and the adaptability of microalgae to polluted environments.

This thesis addresses the energy transition toward sustainable alternatives to fossil-fuels, contributing in advancing the knowledge necessary to make this biotechnological process economically viable.