Food industry wastewater valorization for microalgae cultivation in microplates

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Microalgae are unicellular photosynthetic microorganisms, which have the ability to assimilate organic and inorganic nutrients, minerals and CO_2 from their environment and, at the same time, depending on the cultivation conditions, convert them into high-value products, such as vitamins, pigments, proteins, fatty acids and polysaccharides, (Markou et al., 2014). For this reason, microalgae are characterized as valuable biofuels. Their biomass can be utilized in a wide range of applications in numerous biotechnological industries as it contains various bioactive compounds and pigments (carotenoids and phycobiliproteins). Thus, they are one of the most promising routes for the provision of future foods, feeds, cosmetics, pharmaceuticals, biofuels and wastewater treatment (Jacob-Lopes et al., 2020). Considering their further significant potential for the efficient management of industrial wastewater, the cultivation of microalgae in wastewater represents a vast opportunity to enhance the adoption of the circular bioeconomy model. Municipal, agro-industrial and food wastewaters are some potential residues that have been examined for nutrient recovery using microalgae (Markou et al., 2018). A large number of studies have shown that nitrogen, phosphorus and other nutrients are typically abundant in such wastewaters and regarded as pollutants that must be removed. These nutrients can be utilized from microalgae as they are necessary for their growth. Therefore, they make it possible to remove organic matter and nutrients from the wastewater leading to its reuse (Balasubramaniam et al., 2020).

The objective of the present study was to cultivate three microalgae species in different proportions of food industry wastewaters using microplates as a microreactor platform. Design of Experiments (DoE), specifically an I-optimal mixture design, was employed to optimise the composition of the wastewater cultivation medium for each strain. The goal was to find the optimum set of the proportions of wastewaters for the highest microalgae growth.

Initially, three microalgae strains, two freshwater (i.e. *Chlorella vulgaris*, *Scenedesmus sp.*) and one saline (*Nannochloropsis oculata*) were cultivated autotrophically in a synthetic cultivation medium. The biomass from this native cultivation was then used as inoculum for microalgae screening tests in food industry wastewaters. The cultivation was performed in 24-well plates, and the growth was determined by measuring the optical density (OD) at 680 nm using a microplate reader. Brewery wastewater, expired juice and cheese whey were selected to be the substrates for microalgae cultivation, composing a mix of potentially more than one different wastewater types. In addition to the three wastewaters, water was used as a growth substrate in a lower percentage, so there was a degree of dilution in the wastewater ratios emerging from the experimental design. The I-optimal mixture design was employed to determine the most appropriate proportions of wastewaters for the highest microalgae growth on the 4^{th} day of cultivation (the last day of the exponential growth phase) through a set of 36 runs. A desired space (optimal region) was identified, and an optimum set of wastewater mix was established through the analysis of the graphical contour plots and 3D response surfaces.

Mixture design aided in evaluating the effect of each substrate on the growth of microalgae species. Multi-linear regression (MLR) was employed to fit the mathematical model to the experimental data and predicted R^2 and adjusted R^2 were used to express the quality of the fit. The values of adjusted and predicted R^2 were found to be reasonably close, higher than 0.8, and the adequate precision values were estimated to be greater than 4 (19.96 for *C. vulgaris*, 17.73 for *N. oculata* and 18.04 for *S. sp.*). These results indicated the well-fitted model for predicting the growth of each of the three microalgae in the experimental space. The effects of the ratio of the different wastewaters are graphically represented using graphical contour plots (Fig. 1).

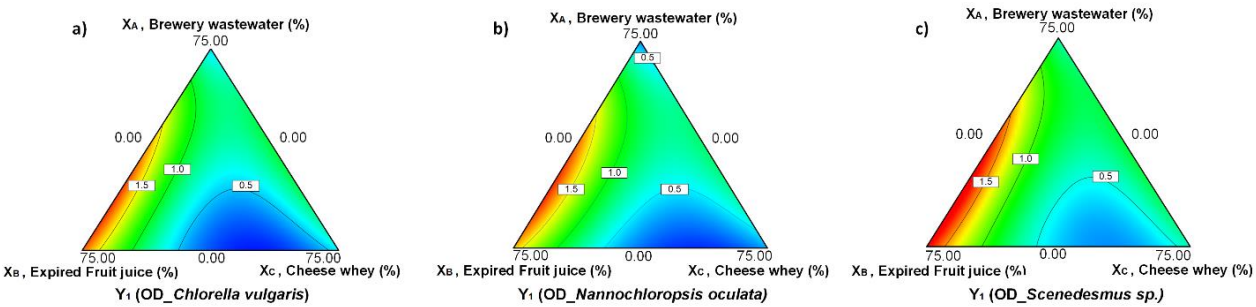

Fig. 1. Mixture contour plots for wastewater ratios for a) *Chlorella vulgaris*, b) *Nannochloropsis oculata* and c) *Scenedesmus sp.*

In Fig. 1 it is evident that the three microalgae grow best in expired juice, followed by brewery wastewater. It is illustrated that with the enhancement of expired juice and the reduction of cheese whey in the substrate mixture, the OD of microalgae tends to increase up to 1.2 for *Chlorella vulgaris*, 1.4 for *Nannochloropsis oculata* and 1.5 for *Scenedesmus sp.* To increase the growth of the three microalgae, the mix of wastewaters should include cheese whey at a value lower than 10% and expired juice at a ratio above 22%. Furthermore, a percentage of brewery wastewater and water between 0 to 50% positively affects microalgae growth. Graphical optimization was performed based on the experimental design, and the Desired Space was determined to establish an optimum set of different ratios of wastewaters satisfying all optimization criteria. Results from optimization are currently analyzed, and the optimum set of wastewater mix for each microalga will be available at the time of the final submission to the conference.

To sum up, the results demonstrate that DoE was a significant tool for finding the appropriate mix of food industry wastewaters that can considerably improve the growth of *Chlorella vulgaris*, *Nannochloropsis oculata* and *Scenedesmus sp*. This biomass could then be utilized to produce bioactive compounds that can be incorporated as additives in cosmetic formulations. Furthermore, the ability to conduct the culture on microplates instead of reactors allows the reduction of the cost, space and time required to evaluate the growth of microalgae, thereby improving the efficiency of screening experiments.

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