

# Improving carbohydrate productivity in a continuous system for microalgal cultivation to biotechnological applications

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Biofuel production is vigorously growing in the twenty-first century and the United States along with Brazil account for about 70% of world production. Among them, bioethanol is the main current application due to its worldwide exploitation (the global ethanol production is comprised between 45 and 50 Mtoe). Thinking of a carbohydrate-rich biomass, microalgal biomass might be a valuable third generation feedstock of bioethanol in the future. In fact, many algal species can accumulate large fractions of carbohydrate under nitrogen limitation. However, in the current literature batch experiments only are reported and a two-step cultivation (biomass cultivation and subsequent starvation) are proposed, to achieve a considerable carbohydrate content (around 50%). This requires a more complex process and affects the stability of production as the biochemical composition of the biomass changes with the growth phase. In addition, from an industrial perspective, steady state production is generally more efficient, has lower cost and is easier to operate.

In this work, two species of microalgae (*Chlorella vulgaris* and *Scenedesmus obliquus*) were cultivated in continuous mode using flat-plate photobioreactors. A limiting nitrogen concentration was supplied to the culture as a method to increase the carbohydrate content in biomass, without affecting too much the overall carbohydrate productivity. The influence of light intensity (150, 300 and 450  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ , and light-dark cycle, summer and winter) and residence time were evaluated.

Concerning the effect of light intensity, it was highlighted that in a continuous system light stress is the main variable affecting the carbohydrate content and productivity, if coupled with a limiting nitrogen supply. It was also evidenced that increasing the residence time is a way to boost nitrogen limitation: the biomass yield on nitrogen noticeably changes with the residence time, thus modifying the elemental composition of the microalgal biomass, resulting in an accumulation of carbohydrates.

By managing these operating variables, it was possible to achieve carbohydrate productivities of 0.37 and 0.9  $\text{g L}^{-1} \text{day}^{-1}$ , for *C. vulgaris* and *S. obliquus*, respectively. Similar or lower results are mentioned as remarkable values in literature, but for batch experiments, enforcing the valuable contribution of this work.

The carbohydrate production was also assessed under light-dark cycle: it was verified that the biomass and carbohydrate production changes significantly during the day, decreasing in dark periods (15-25% for biomass and 20-40% for carbohydrates losses), as expected, due to respiration.