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Rhodosporidium toruloides and *Tetradesmus obliquus* Populations Dynamics in Symbiotic Cultures, Developed in Brewery Wastewater, for Lipid Production

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Abstract

In this work, primary brewery wastewater (PBWW) and secondary brewery wastewater (SBWW) separately, or mixed at the ratios of 1:1 (PBWW:SBWW) and 1:7 (PBWW:SBWW), with or without supplementation with sugarcane molasses (SCM), were used as culture media for lipid production by a mixed culture of the oleaginous yeast *Rhodosporidium toruloides* NCYC 921 and the microalgae *Tetradesmus obliquus* (ACOI 204/07). Flow cytometry was used to understand the dynamics of the two micro-organisms during the mixed cultures evolution, as well as to evaluate the physiological states of each micro-organism, in order to assess the impact of the different brewery effluent media composition on the microbial consortium performance. Both brewery wastewaters (primary and secondary) without supplementation did not allow *R. toruloides* heterotrophic growth. Nevertheless, all brewery wastewater media, with and without SCM supplementation, allowed the microalgae growth, although the yeast was the dominant population. The maximum total biomass concentration of 2.17 g L^{-1} was achieved in the PBWW mixed cultivation with 10 g L^{-1} of SCM. The maximum lipid content (14.86% (w/w DCW)) was obtained for the mixed culture developed on SBWW supplemented with 10 g L^{-1} of SCM. This work demonstrated the potential of using brewery wastewater supplemented with SCM as a low-cost culture medium to grow *R. toruloides* and *T. obliquus* in a mixed culture for brewery wastewater treatment with concomitant lipid production.

Introduction

Microalgae/yeasts mixed cultures have been considered an auspicious approach for lipid and carotenoid production, presenting many benefits over single cultures, such as higher biomass, lipid and carotenoids production [1, 2]. The advantages of the symbiotic cultures result from the synergistic relationship between the two micro-organisms [1].

When grown in mixed cultures, yeast and microalgae benefit from the heterotrophic/autotrophic nutritional modes that have complementary requirements. Yeasts produce carbon dioxide during their respiration, which can be used as carbon source for the autotrophic metabolism of the microalgae, while the microalgae produce oxygen, essential for the yeast metabolism [1, 2]. In this way, it is possible to avoid carbon/oxygen limitations for yeast/microalgae populations, respectively. Furthermore, other advantages such as metabolite exchanges and medium pH auto-adjustment are observed in mixed cultures [1]. Therefore, yeast and algae when cultivated in mixed cultures produce intracellular compounds more efficiently with potential commercial interest, such as lipids and carotenoids [3, 4].

Low-cost substances, such as wastewater, have been extensively used as growth media for microbial growth, aiming at the concomitant wastewater treatment and interesting intracellular products, in order to reduce the microbial process costs [1]. This strategy can be applied to yeast and microalgae symbiotic cultures for intracellular lipid production, for biodiesel purposes. In addition, microalgae are very efficient in ammonia, phosphorus, and heavy metal removal

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