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ORIGINAL PAPER

Effect of brewery effluent inhibitors on *Rhodotorula toruloides* NCYC 921 cells grown in pure and mixed cultures at pH 4 and 6

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Abstract
The presence of inhibitor compounds in the culture medium can cause severe effects on the microorganisms cells. Brewery wastewaters present organic acids (acetic, propionic and butyric acids) which can severely affect yeast cells metabolism, when grown in pure cultures, although in mixed cultures they are able to develop. To understand the physiological changes on *Rhodotorula toruloides* (formerly *Rhodosporidium toruloides*) cells when fermenting in the presence of the organic acids present in brewery wastewater, pure and mixed cultures with the microalga *Tetradesmus obliquus* were performed in a synthetic medium containing the same organic acids concentrations that are present in brewery wastewater at pH 4 and 6. It was concluded that, at pH 4, the organic acids effects in the yeast cells were much more toxic than at pH 6. Moreover, mixed cultures can be an advantage over heterotrophic pure cultures as the microalga is able to contribute for the consumption of potential inhibitors for the yeast.

Keywords *Rhodotorula toruloides* · *Tetradesmus obliquus* · Organic acids · Inhibitors · Flow cytometry

Introduction

Oleaginous microorganisms such as yeasts and microalgae can be used as biodiesel feedstocks, as they are capable of accumulating between 20 and 80% lipids of their dry cell weight (Dias et al. 2020a). However, the cost of the biodiesel produced from oleaginous microorganisms is still not economically sustainable, since production costs are still high (Dias et al. 2015; Zhang et al. 2017). To reduce the microbial biodiesel production costs, it is crucial to use low-cost substrates which can be used as feedstock for oleaginous microorganisms' growth. Brewery wastewater is an example of an effluent that has been successfully used as feedstock for heterotrophic or autotrophic oleaginous microorganisms such as yeasts or microalgae, respectively, since it usually has high organic and inorganic loads that can be used as feedstock by these microorganisms (Schneider et al. 2013; Marchão et al. 2018; Dias et al. 2020a, 2022a, 2022b). However, industrial effluents can contain toxic compounds that can inhibit microorganisms growth (Guerra, 2005).

Dias et al. 2022c identified the presence of three organic acids in primary brewery wastewater (PBWW): acetic (HAc), propionic (HProp) and butyric (HBut) acids at a concentration of 0.5, 0.4 and 1.0 g L^{-1}, respectively. With the addition of sugarcane molasses as carbon source for heterotrophic growth, this concentration rose to 1.1, 1.0 and 1.0 g L^{-1} of HAc, HProp and HBut, respectively. In the secondary brewery wastewater (SBWW) media no weak acids were detected. However, in the SBWW medium supplemented with sugarcane molasses, HAc was detected at a concentration of 0.8 g L^{-1} and HProp only at residual concentrations (0.07 g L^{-1}) (Dias et al. 2022c). The increase in the organic acids concentrations, after the effluent supplementation, was attributed to the addition of sugarcane molasses, since in the manufacturing process, several inhibitor compounds can be present in the final sugarcane molasses such

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