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Impact of brewery wastewater inhibitors in pure and mixed cultures of the yeast *Rhodosporidium toruloides* NCYC 921 and the microalga *Tetradesmus obliquus* ACOI 204/07

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ABSTRACT

Brewery wastewater (BWW) is an appealing low-cost substrate for the production of single cell oils by oleaginous microorganisms. However, it may contain inhibitor compounds that may affect the microbial metabolism. This work investigated, for the first time, the presence of potential inhibitor compounds in primary brewery wastewater (PBWW) and secondary brewery wastewater (SBWW) for the pure and mixed cultivation of the yeast *Rhodosporidium toruloides* NCYC 921 and the microalga *Tetradesmus obliquus* ACOI 204/07. Three organic acids (OrgAc) were identified in the brewery effluents (acetic, propionic and butyric acids). Yeast and microalga pure and mixed cultivations were performed in PBWW and SBWW in order to understand the behaviour of the microorganisms, individually and together. Flow cytometry (FC) was used to monitor each microbial population during the mixed cultivations, and to study the yeast and microalga cell viability throughout all cultivations. The yeast cells in pure cultures grown in both effluents were severely affected by the OrgAc presence confirmed by the cell stress results obtained by FC. However, in the mixed cultures, the yeast cells were able to develop, and the levels of stress conditions were considerably lower. Only in microalga pure and mixed cultures efficient OrgAc removal was observed.

1. Introduction

Brewery wastewater (BWW) has been proposed as an attractive low-cost substrate for microbial lipids (single cell oils - SCO) production [1–3]. For each liter of beer produced, from 3 to 10 L of wastewater are generated [3,4]. BWW have been described to be rich in organic compounds, such as sugars, soluble starch, proteins, phosphates, ammonia, ethanol, nitrate, nitrogen and phosphorus. Due to this, brewery wastewaters can be used as culture medium for autotrophic metabolism [5,6] or, for heterotrophic growth, especially when supplemented with an external carbon and nitrogen source, such as sugarcane molasses and urea, since some BWW have been described to contain low carbon and nitrogen concentrations to allow the efficient microorganisms growth [3,7]. This strategy allows reducing the culture medium cost, as well as obtaining high lipid contents and other high value compounds which can make the process profitable [3]. Additionally, the use of industrial effluents/wastes as substrates for microbial growth can be a strategy to treat them based on the circular economy rules [8].

Although the use of industrial effluents can be an appealing approach, it has been reported that they can contain toxic compounds, such as heavy metals, organically bound metals and metalloids, trace organic pollutants, polychlorinated biphenyls, pesticides, detergents, chemical carcinogens, among others [9], that can inhibit microbial metabolism. For instance, the presence of heavy metals in the wastewater can lead to displacement and/or substitution of essential metal ions in biomolecules, blockage of functional groups in biologically important molecules such as enzymes, disruption of protein structure and membrane integrity or reduction of growth and photosynthetic activity in microalgae [10].

Also, when hemicellulosic hydrolysates or derivates are used as carbon source for heterotrophic growth, there are several potential inhibitors that can be present in the medium such as aliphatic acids (acetic

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