A continuous bioprocess for 2,3-butanediol production and separation through crude glycerol and corn steep liquor valorisation

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ABSTRACT

Biodiesel production, having a constantly growing industrial application the last many years, generates biodiesel derived-glycerol as the main side product of the process [1]. The aim of the present study was to optimize the bioprocess, concerning the fermentation of biodiesel-derived glycerol to 2,3-butanediol (BDO), by a very promising bacterial strain (viz., Klebsiella oxytoca ACA-DC 1581) [2].

The culture medium was optimised, while both digestate and corn steep liquor, the main by-products of the biogas and corn industries, respectively, successfully served as the sole source of nitrogen, contributing to completely replace more expensive sources. It was shown that the absence of thermal treatment of the substrate had no negative impact on the growth of the microorganism and the production of BDO, which allowed the development of a non-aseptic bioprocess. The volumetric mass transfer coefficient (kLa) was optimized to 70.5 1/h and in all cases *K. oxytoca* produced almost 70 g/L BDO. After optimization and during the first 48 h of the most successful fed-batch fermentation, BDO productivity increased 2.5 times (to *c.* 1.10 g/L/h), the yield of BDO production to glycerol consumption ($Y_{BDO/Glv}$) was 0.46 g/g, while the ratio of BDO to the sum of metabolic products was 96.3%, indicating a high selectivity of the bioprocess for BDO. In the next step, the upscaling of the optimized bioprocess was successfully carried out in a pilot-scale bioreactor (250L) at Verd S.A. plant.

Various dilution rates (D) were tested in continuous cultures, with the D of 0.04 1/h showing the highest values. More specifically, BDO productivity reached 2.10 g/L/h, glycerol consumption rate was 3.14 g/L/h and $Y_{BDO/Glv}$ was 0.45 g/g, while the BDO titer was 50.5 g/L. This is the first time that continuous production of BDO from glycerol has been reported. Finally, a downstream process was developed in which salting-out extraction (SOE) was applied to recover 90% BDO from the fermentation broth [3]. The partition coefficient of the unconsumed glycerol needs further investigation as it was equally distributed in both phases. These results are among the highest in the international literature and demonstrate the feasibility of this fermentation on an industrial scale.

KEYWORDS: Industrial biotechnology, Downstream, Bio-based chemicals, Waste valorisation, *Klebsiella oxytoca*

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