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Single-cell Biomass Production by Rhodosporidium Toruloides Growing on Crude Glycerol with Process Water Being Partially Replaced by Olive-mill Wastewaters

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Abstract

The constantly rising demand for utilization of conventional fuels, the subsequent accompanying environmental issues implicated (i.e., greenhouse gas emissions) and the constantly rising price of the non-renewable fuels (i.e. petrodiesel) have resulted in the significant need for utilization of biofuels in commercial-scale operations. One of the most important biofuels implicated is biodiesel (viz. mostly fatty acid methyl-esters). The continuous increase in biodiesel production makes mandatory the exploitation / valorization of the main by-product of the processes, viz. crude glycerol. On the other hand, a major environmental problem deriving from olive oil production and mostly affecting the Mediterranean countries, is the generation in high quantities of the highly toxic olive mill wastewater (OMW), while on a recent development, OMWs can serve as simultaneous process water and substrate in order to “dilute” “dense” resides like crude glycerol. In our study, OMWs containing initial phenolic quantities ≈2.6 g/L and low initial sugar concentration (≈6.0 g/L) served as process waters (OMWs diluted to a ratio of 50% v/v, therefore phenolic compounds into the medium were ≈1.3 g/L), in which biodiesel-derived glycerol (purity ≈75% w/v) was used as carbon source (initial glycerol concentration ≈60 g/L). The non-conventional and non-pathogenic yeast *Rhodosporidium toruloides* (strain NRRL Y-27012) was used as microbial cell factory, in order to produce dry cell weight (DCW) from these residues in submerged flask fermentations. Trials were performed under nitrogen excess conditions that were employed in order to enhance the production of DCW and not to favor the synthesis of cellular lipids. In media supplemented with OMWs and despite the presence of inhibiting compounds (i.e., phenols), DCW up to 33.2 g/L was synthesized (yield on glycerol consumed ≈0.58 g/g). In the “blank” experiment (no OMWs added - control) DCWmax =27.5 g/L was produced. Moreover, the assimilation rate of glycerol in media supplemented with OMWs was (interestingly) higher than that of the control. Cellular lipids and polysaccharides in DCW values varied through the cultures. In the experimental point with the highest DCW achieved, lipid in DCW for both trials was 5-7% w/w, while remarkably when OMWs supplemented the media, higher polysaccharide values were recorded (22% w/w against 10% w/w for the control). In the same sampling point, protein content was ≈38% w/w for the “blank” experiment and ≈32% w/w for the trial where OMWs

supplemented the medium. Finally, cultures in the OMW-based media, were accompanied by ≈55% w/w dephenolization and ≈40% decolorization. Concluding, blends of OMWs and crude glycerol were successfully used by *R. toruloides*, appreciable quantities of DCW were produced and interesting detoxification (removal of phenols and color) of the medium occurred.

Keywords: Crude glycerol, Olive-mill wastewaters, Yeast lipids, Yeast polysaccharides, Yeast proteins

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