Biomass and Citric Acid Production by *Yarrowia lipolytica* Cultivated on Olive Oil Mill Wastewater-based Media

Dimitrios Sarris\(^a\), Maria Galiotou-Panayotou\(^a\), Michael Komaitis\(^b\), Seraphim Papanikolaou\(^a\)*

\(^a\) Laboratory of Food Microbiology and Biotechnology, \(^b\) Laboratory of Food Chemistry, Department of Food Science and Technology, Agricultural University of Athens, Greece

*Corresponding author: spapanik@aua.gr*

Olive oil mill wastewaters (OMWs) produced annually in tremendous quantities (more than 30 million m\(^3\) per year) and containing phenol (toxic) compounds and sugars (in same cases in very high concentrations) are one of the most difficult to treat effluents. The need to limit (by detoxification and decolorization) the environmental pollution as well as to produce high value products (citric acid and biomass) from enriched OMWs by cost efficient biological treatment methods was the aim of the present study. In the present investigation a *Yarrowia lipolytica* strain (ACA-YC 5033) was cultivated on OMW-based media containing initial phenol compounds of 2.10 g/L in both nitrogen and carbon-limited flask experiments. Commercial glucose, an industrial low-value product, was added in both media in order to achieve an initial sugar quantity of 27±2 g/L (a typical concentration of reducing sugars in OMWs), while salts were also added. (NH\(_4\))\(_2\)SO\(_4\) and yeast extract were the nitrogen sources. Blank experiments (without OMW addition) were also carried out. In carbon-limited fermentations, significant quantities of biomass (X\(_{\text{max}}\)=10.0±1.0 g/L) were produced in both instances. The presence of phenol compounds into the waste did not inhibit microbial growth (in contrast biomass concentration presented surprisingly slightly higher values in the experiment with OMW). Intracellular lipids of 1.0±0.05 g/L (around 10%, wt/wt, in dry matter) were produced in both instances. Given that culture was done in carbon-limited media, naturally, no citric acid production was observed. Furthermore, in the experiment in which OMW was added, no decolorization was observed while, concerning detoxification of the residue, phenol compounds decreased no more than 5%, wt/wt (phenol compounds in the end of fermentation 2.0 g/L). In nitrogen-limited fermentations, naturally, 45 to 62% less biomass was observed (X\(_{\text{max}}\)=5.5±1.0 g/L). Maximum biomass concentration clearly decreased in the OMW experiment (to around 25%). Intracellular lipids content seemed to increase due to the nitrogen limitation (total lipids 14-19%, wt/wt, in dry matter), while somehow higher concentrations of cellular lipids were observed in the experiment with the presence of OMW. Citric acid (Cit) in relatively satisfactory concentrations was produced, while OMW seemed not to inhibit its production (Cit\(_{\text{max}}\)=18.17±0.5 g/L in OMW-based media and 18.85±0.5 g/L in blank experiments). Finally, the waste was satisfactorily decolorized up to 56% while a non-negligible phenol removal was observed (around 13%, wt/wt – final phenol compounds concentration after fermentation 1.83 g/L).