



Olive mill wastewater biodegradation for bacterial lipase production using a response surface methodology

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Abstract

Olive mill wastewater (OMW) represents the effluent generated during the olive oil extraction process. OMW, as an oil-rich residue, provides a source of lipase-producing microorganisms and a source of complex nutrients that is potentially suitable to be applied in bacterial lipase production. This study focused on isolating actinobacteria able to produce extracellular lipases with OMW as the sole carbon source. Thirteen isolates of actinobacteria have been obtained and screened for their lipase activity using rapid plate detection methods. Then, the extracellular lipolytic activity was monitored under shaking flask conditions, and the titrimetric method was applied for lipase activity determination. Maximum lipase activity was detected for the SC1 strain (5.33 U/mL), which was then identified as a *Streptomyces* species. To enhance lipase production using the SC1 strain, Box-Behnken design (BBD)-based response surface methodology (RSM) has been adopted, by varying incubation time (days), pH, temperature (°C), inoculum size (spores/mL), and initial OMW concentration (% v/v). An accompanying analysis of variance (ANOVA) was carried out, and the production of lipase was reported by a mathematical equation according to the factors. Non linear regression equations with significant R^2 and p -values were used to depict individual term, interaction, and square effects on lipase production. Individual term, interaction, and square effects on lipase production were shown using nonlinear regression equations with significant R^2 and p -values. The optimization results obtained showed that the optimal lipase activity was achieved after a 9 days incubation time at pH 4, with an inoculum size of 1.7×10^7 (spores/mL), incubated at a temperature of 30°C using 6% (v/v) OMW as the sole carbon source.

Keywords Olive mill wastewater · Lipase activity · Actinobacteria · Response surface methodology · Biodegradation

1 Introduction

Olive mill wastewater (OMW) is produced annually in large quantities around the Mediterranean basin. Olive mill wastewater is considered a toxic pollutant, especially when discharged into aquatic environments [1]. This toxicity is mostly because it contains fats and oils that have previously

undergone physical and chemical treatments and are now in a dispersed form. Recently, the biological treatment process is a more efficient method to eliminate fats and oils by degradation to miscible molecules. Oil mill wastewater can be considered a resource that contains simple and complex carbohydrates, proteins, and minerals and therefore can be used for fermentation processes. Furthermore, the wastewater contains lipids and residual oil, a fact that makes the effluent the ideal medium for the growth of lipase-producing microorganisms [2].

Lipases (triacylglycerol acyl hydrolase; E.C. 3.1.1.3) are enzymes that catalyze the hydrolysis and synthesis of esters from long-chain glycerol and fatty acids. For decades lipases have been employed in various industries to hydrolyze fats and catalyze several useful reactions including esterification and transesterification—applications typically applied in leather industries [3].

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