

Alternative feedstocks for high-quality biodiesel: Lipid production from eucalyptus bark hydrolysate by *Yarrowia lipolytica* W29 using different cultivation modes

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Abstract: Microbial lipids produced by yeasts from lignocellulosic biomass are a promising feedstock for the biodiesel industry, providing a renewable energy source as an alternative to traditional fossil fuels. This study investigated the potential of *Yarrowia lipolytica* W29 to produce lipid-rich biomass from undetoxified sugar-concentrated eucalyptus bark hydrolysate (EBH). The lipid concentrations achieved in batch cultures (13.4 g L^{-1}) were the highest for wild-type *Y. lipolytica* strains in lignocellulosic hydrolysates. Different two-stage cultivation modes (repeated batch, continuous-feeding fed-batch, and pulse fed-batch) were studied to enhance biomass and lipid production. The cell and lipid mass was higher in pulse fed-batch and continuous-feeding fed-batch cultures than batch cultures. Production of citric acid, a side product of industrial interest, was improved in the continuous-feeding fed-batch culture. Microbial lipids produced by *Y. lipolytica* W29 were highly unsaturated and mainly composed of oleic acid (50% to 53%). The estimated properties of the biodiesel that would be obtained from these intracellular lipids would meet the international biodiesel standards EN 14214 and ASTM D6751. This study demonstrates the feasibility of using EBH for *Y. lipolytica* lipid production and promotes the sustainable production of high-quality biodiesel from lignocellulosic feedstocks. © 2025 Society of Industrial Chemistry and John Wiley & Sons Ltd.

Key words: eucalyptus bark hydrolysate; microbial lipids; citric acid; cultivation modes; biodiesel