Sustainable production and interfacial properties of amphisin biosurfactant for flotation applications

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The growing demand for sustainable and cost-effective biosurfactant production has intensified interest in utilizing industrial waste streams as fermentation substrates. In this study, crude glycerol derived from biodiesel and soap manufacturing was evaluated as a carbon source for amphisin production by *Pseudomonas fluorescens* DSS73. Among the tested substrates, glycerol originating from soap production supported the highest biomass formation and amphisin yield (4.62 $g \cdot L^{-1}$), highlighting its suitability for biotechnological applications.

To further enhance production efficiency, a Box–Behnken experimental design was applied to optimize key process variables, including glycerol concentration, corn steep liquor (CSL) concentration, and cultivation time. Statistical analysis revealed that all three factors significantly influenced amphisin biosynthesis, with glycerol concentration exerting the strongest effect. The developed quadratic model demonstrated excellent predictive capability ($R^2 = 0.9999$). Under optimized conditions (70.9 $g \cdot L^{-1}$ glycerol, 29.7 $g \cdot L^{-1}$ CSL, 5.9 days), amphisin production reached 7.51 $g \cdot L^{-1}$, closely matching the predicted value.

Comprehensive physicochemical characterization of purified amphisin confirmed its strong surface activity and complex interfacial behavior. The biosurfactant exhibited a low critical micelle concentration (4.7×10^{-6} $mol \cdot dm^{-3}$) and reduced surface tension to 39.2 $mN \cdot m^{-1}$. Potentiometric titration revealed two dissociation constants ($pKa_1 = 2.35$ and $pKa_2 = 5.29$), indicating pH-dependent ionization. Spectroscopic and electrokinetic analyses further confirmed the transition from neutral to anionic forms with increasing pH. Additionally, dynamic and equilibrium surface tension studies, supported by diffusion-based modeling, demonstrated that amphisin adsorption is initially diffusion-controlled but becomes kinetically limited at higher surface coverage. Molecular dynamics simulations revealed the formation of non-spherical, discoidal micelles with complex internal organization.

Overall, this work demonstrates an efficient and sustainable strategy for amphisin production from waste glycerol and provides detailed insight into its interfacial and aggregation properties, supporting its potential in flotation and environmental applications.

Keywords:
amphisin; biosurfactants; crude glycerol; waste valorization; Pseudomonas fluorescens; process optimization; Box–Behnken design; surface activity

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