

Optimizing Biosurfactant extraction yield from Corn Steep Liquor: A Strategic Approach for Cost-Effective Pilot-Scale Biopesticide Product

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The purpose of this research is to protect agricultural production and ecological environment as well as prevent and eliminate pests reducing hazards from agro-pesticides by the inclusion of biosurfactants extracted from corn steep liquor (CSL) in pesticide formulations. In previous works (Vecino et al., 2014, 2015) it was demonstrated that CSL, a spontaneously fermented stream of corn milling industry, can be a direct source of a biosurfactants with antimicrobial and solubilizing properties, of high interest in pesticide formulations. The production of this biosurfactant a lab scale was carried out successfully by liquid-liquid extraction with ethyl acetate with an extraction yield about 11 g of biosurfactants/kg of CSL. However, to be competitive with chemical surfactants it is necessary to increase the extraction yield of this biosurfactant extract obtained from CSL. Thus, the aim of this work was to explore how to increase the extraction yield of biosurfactants from CSL to face a cost competitive pilot scale production of biosurfactants. For that CSL was diluted at different concentrations in water previously to the extraction with ethyl acetate following the procedure described in the patent WO 2014/044876 (Moldes et al., 2014), and the ratio of organic solvent also was varied between 1:1 and 1:3 (CSL:ethyl acetate). The dependent variables selected in the study were the extraction yield, surface tension and antimicrobial activity of the biosurfactant extract. The yield of the extraction process was evaluated based on the dry weight of the extract, the surface tension of these dilutions was measured using a K20 Easy Dyne Tensiometer supplied by Krüss GmbH (Hamburg, Germany), allowing the use of the Wilhelmy plate method and the antimicrobial activity of the biosurfactant was established against *Aspergillus* by measuring the reduction on colony-forming unit (CFU) in presence of this biosurfactant extract. After determining optimal conditions at lab scale with a working volume of 1 L, the research progressed to pilot-scale production, utilizing a working volume of 1000 L per batch.

The findings demonstrated that extraction yield of biosurfactant increased with the concentration of CSL in the aqueous phase and the more favorable CSL: ethyl acetate ratio was 1:3 (see **Figure 1**).

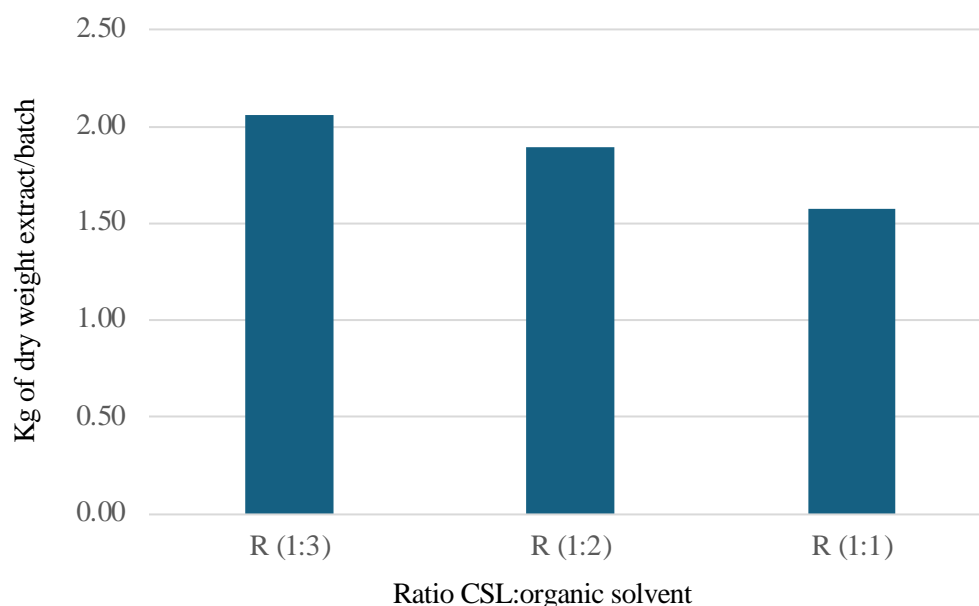


Figure 1. Estimation of kg of biosurfactant extract produced at pilot scale for a working volume of 1000 L.

Remarkably, the biosurfactant produced at the pilot scale exhibited analogous characteristics to those produced at the lab scale. The cost of biosurfactant produced a pilot scale under the selected conditions was estimated in 110 €/Kg. Considering the demonstrated antimicrobial properties of this biosurfactant extract at concentrations of 1 g/L, it can be concluded that could be feasible to formulate a biopesticide with a reduced concentration of copper oxychloride. Previous studies have shown that the biosurfactant extract enhances also the solubilization of copper oxychloride in water (López-Prieto et al., 2020). Hence, considering the outcomes presented in this study along with insights gleaned from prior research, it is evident that biosurfactants derived from CSL enable the formulation of a biopesticide with enhanced efficacy and reduced dependence on copper oxychloride, that according to the classification provided by companies to European Chemical Agency ECHA in the classification, labelling and packaging (CLP) of substances, this substance used as agrochemical is toxic if swallowed, is very toxic to aquatic life with long lasting effects and is harmful if inhaled.

After the optimization of biosurfactant extraction from CSL, the manufacturing expense for the new biopesticide, involving biosurfactants derived from CSL is projected to be under 0.5 € per liter of the end aqueous formulation product.

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