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Interestingly, under both biotransformation pathways the SFA content was similar. Finally, the biodiesel properties of the corresponding FAMEs, FAEEs and FABEs were theoretically estimated by using the FA profiles with the help of derived empirical formulas. Then, the data were compared by international standards such as EN14214 and ASTM D6751. The analysis showed that the oleaginous biomass from A. niger MYA 135 could be used as potential feedstock for biodiesel production. S: Saturated; PU: Poly unsaturated; MU: Mono unsaturated; M: Methyl; E: Ethyl; B: Butyl; Es: esters.

Keywords: Biotransformation, Industrial residues, Microbial lipid, Submerged fermentation, Fungal morphology

Methods: Folch method, Sudan Black staining, Thin layer chromatography, Gas chromatography, Experimental design

BT-07

Sustainable two-step multienzymes-assisted aqueous processing of soybean flour yielding free oil, proteins and carbohydrates: enzymatic cocktail production, characterization and potential reutilization

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Oil production by enzyme-assisted aqueous extraction (EAAE) may be a viable, safer, and environmentally friendly alternative to the traditionally hexane extraction. Enzymes are necessary to improve oil extraction yields, as they break down the cell wall and protein barriers surrounding the oil bodies. Cellulases, phospholipases, and proteases are widely used to overcome these barriers. However, the use of commercial enzymes makes this process more expensive. Thus, in order to create a more efficient and sustainable system, the main objective of this work was to synthetize and characterize a multi-enzymatic cocktail obtained by submerged fermentation from solid and liquid wastes generated during an EAAE process using *Brevibacillus agri* E12. Firstly, a genome-wide identification of coding sequences corresponding to cellulases, proteases, and phospholipases activities from *B. agri* E12 was done. Then, the microbial culture supernatant obtained after 24 h of incubation from the Luria-Bertani (LB) reference medium was used in a EAAE process at pH 9 and 50 °C. The solid and liquid residues of

this process were employed to formulate the R1 culture medium (liquid fraction: 5 %, v/v; solid fraction: 10 %, p/v). The stability of the culture supernatants obtained from R1 and LB media were assayed under the EAAE conditions (50 °C at different pH values during 24 h). The hydrolytic enzymes showed residual activity above 60 %. Additionally, the optimal temperature and pH for each hydrolytic activity were as follows: for cellulase, 40 °C, pH 5; for phospholipase, 37 °C, pH 7; and for protease, 60°C, pH 9. Finally, as proof of concept, two-step EAAE were carried out using culture supernatants from R1 and LB media. Step 1: solid-liquid ratio of 1:20, at 50 °C, for 24 h, and under different pH values (5, 7 and 9). Step 2: solid-liquid ratio of 1:20, at 50 °C, for 4 h, and at pH 9. The highest yields with respect to soybean flour were obtained at pH 9 (LB: 17.17 ± 0.45 %; R1: 18.09 ± 0.53 %; Hexane: 21.05 ± 1.02 %). Besides, the fatty acid profile of the oil extracted by EAAE using the multienzymes cocktail from R1 medium was similar to that extracted by hexane. The results showed that the multienzymes cocktail recycling could also be viable as after EAAE at pH 9 biocatalysts retained over 78% of their activities. In addition, the liquid fraction obtained after EAAE at pH 9 contained the same concentration of carbohydrates (LB: 0.91 ± 0.07 g/L; R1: 1.04 ± 0.03 g/L) and proteins (LB: 5. 49 ± 0.31 g/L; R1: 4.83 ± 0.25 g/L) (means are not significantly different). The electrophoretic profile of proteins in the liquid fraction showed the presence of several peptides with molecular weights less than 25 KDa when the EAAE was carried out at either pH 7 or 9. Thus, under the circular economy concept, solid and liquid wastes generated from a EAAE process can be revalorized via enzyme production allowing a viable, efficient, and sustainable soybean oil EAAE process.

Keywords: Soybean oil, Aqueous extraction, Hydrolytic enzymes, Waste valorization, Sustainable technologies,

Methods: Oil extraction, Enzymatic determinations, Polyacrylamide gel electrophoresis, Thin layer chromatography, Gas chromatography

BT-08

EXPANDING THE BIOCATALYTIC TOOLBOX WITH A NEW REDUCTASE FROM LEPTOSPIRA BIFLEXA

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The search for ecofriendly ways to synthesize chemical substances has led to the incorporation of biocatalysts, such as pure enzymes, whole microorganisms or plant-derived materials, into synthetic