Evaluation of Ionic Liquids and Deep Eutectic Solvents as green solvents for activity of ligninolytic enzymes of actinobacteria isolated from Brazilian Caatinga

Gutierres F. C.[1]; Okamoto D. N.[1]; Chagas J. R.[1]; Longo Jr L. S.[1]; Vasconcellos S. P.[1]

[1]Department of Pharmaceutical Sciences, Universidade Federal de SãoPaulo (UNIFESP), R. São Nicolau, 210 - Diadema, SP, Brazil, Zip Code 09913-030.

UNIFESP – Federal University of São Paulo – Brazil

Enzymatic hydrolysis of plant carbohydrates is an eco-friendly alternative to reduce CO_2 emissions. However, after this process, it has an excess composed of cellulose, hemicellulose and lignin. One of the greatest problems is how to solubilize the lignin. Ionic Liquids (Il's) were evaluated as carrier solvents of lignin monomers (phenylpropanoids), in the same moment that actinobacteria were evaluated about their lignin peroxidase activities. Enzymatic broth characterized as bacterial supernatant of four (4) actinobacteria isolated from Brazilian Caatinga were evaluated under veratryl alcohol. Enzymatic kinetic values were found in the order of 10^{-4} to 10^{-7} mM for K_{Mapp} and 55 to 76 min^{-1} for $V_{maxapp.}$ Evaluating laccase abilities, the oxidation of guaiacol was measured. Laccase kinetic values were 10^{-5} to 10^{-8} mM for K_{Mapp} and 1 to 21 min^{-1} for V_{maxapp}. Besides, the toxicity of ionic liquids, $[C_8C_1Pyrr][NTf_2]$ (IL-1), $[C_8C_1Im][NTf_2]$ (IL-2), $[C_6(C_1Im)_2[(NTf_2)_2]$ (IL-3) and $[C_8(C_1Im)_2][(NTf_2)_2]$ (IL-4), as well deep eutectic solvents (DES) ClCH/Urea (DES-1) and ClCH/Ethyleneglycol (DES-2), the following concentrations were evaluated: 0.01%, 0.1%, 1% and 2%. Bacterial growth was analyzed by OD_{600} measurement and cell viability using MTT reaction. It was found that all the evaluated actinobacteria could grow in 0.01% in all IL's and DES. In the presence of 2% IL-3, DES-1 and DES-2, the viability was 100% for AC16. Using IL-4 and DES-2 only AC22 could survive, while in DES-2 and IL-3 the growth was confirmed for AC153 and AC159, respectively. In this context, purified, lignin was solubilized in IL's and DES, following to monitoring analysis using MALDI-TOF, LC-MS and HPLC. These experiments are now in advance, but the results obtained until this moment are instigating ourselves to believe ionic liquids and DES could be potential solubilizing agents to be used as green carrier for enzymatic reactions that are looking the biotransformation of lignocellulosic biomass as substrate for bioethanol production or bioactive compounds from lignin polymer as raw material.

Keywords: Actinobacteria, Bioethanol, Caatinga, Laccase, Lignin Peroxidase.