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RESUMO: Although the area of biomass pretreatment has been improved in the last decades, there are challenges regarding the process scale up, the development of continuous processes and the formation of biomass conversion inhibitors. An option to circumvent these shortcomings would be the use of extrusion for biomass pretreatment. This study evaluated the pretreatment of sugarcane biomass in a twin-screw extruder by taking into consideration the use of additives such as water, glycerol, ethylene glycol, and Tween® 80, the selected additive load, the process temperature, rotating speed, number of passes and screw design. Results showed that using a biomass:glycerol ratio of 1:0.75 for bagasse and 1:0.53 for straw the pretreatment efficiency, measured by the biomass hydrolysis yields, was increased after multiple extrusion passes and, more importantly, the insertion of a reverse element in the screw configuration, resulting in a straw hydrolysis yield of 68.2%. These results indicate clearly the potential of extrusion for sugarcane biomass pretreatment.