The high-level production of enzymes is an important bottleneck on second generation ethanol production. In this study, we present our successful experience on using *Aspergillus* high-expression system (pEXPYR), which enables target secretion of recombinant proteins. We characterized the operation mode of a core set of biomass conversion enzymes, two fungal GH7 cellobiohydrolases (AfCbhA and AfCbhB), a GH11 xylanase (PfXyn) and a GH54 arabinofuranosidase (AnAbf). AfCbhA and AfCbhB have similarity in catalytic domain, but only AfCbhA contains a carbohydrate binding domain. Modeling studies identified the typical tunnel-like catalytic active site on both Cbhs, but only AfCbhA shows an additional loop that appears to obstruct the substrate-fitting channel. Biochemical studies suggest that while AfCbhB only hydrolyzes loose chains of cellulose, AfCbhA releases new cellulosic chain from the cellulosic fiber. Additionally, the synergistic breakdown of wheat and rye arabinoxylan as well as sugarcane bagasse by PfXyn and AnAbf were comprehensive determined. This work contributes to the formulation of optimized cocktails for bioethanol production, aiming at to avoid excess on protein loads or unnecessary enzymatic activities.

Keywords: pEXPYR, synergism, *Aspergillus*.

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