INDIAN INSTITUTE OF TECHNOLOGY GUWAHATI
SHORT ABSTRACT OF THESIS

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Programme of Study	:	**Ph.D.**

Thesis Title: "**Structure and functional analysis of a recombinant endo-*β*-1,4-xylanase (*Ac*Xyn30B_12) from *Acetivibrio clariflavus* DSM 19732 and its application in deinking of waste papers**"

Name of Thesis Supervisor(s)	:	**Prof. Arun Goyal**
Thesis Submitted to the Department/ ~~Center~~	:	**Biosciences and Bioengineering**
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SHORT ABSTRACT

A gene encoding an endo-β-1,4-xylanase of glycoside hydrolase family 30 (GH30) was identified from *Acetivibrio clariflavus* DSM 19732 genome and designated as AcXyn30B_12. *Ac*Xyn30B_12 was successfully cloned, expressed in *E. coli* BL21 (DE3) and purified by immobilized metal-ion affinity chromatography followed by SEC. The SDS-PAGE analysis of *Ac*Xyn30B_12 revealed a molecular mass, ~74 kDa. *Ac*Xyn30B_12 exhibited highest specific activity against partially acetylated birchwood xylan at optimal conditions, 70°C and pH 5.5. *Ac*Xyn30B_12 retained over 80% activity across a broad pH range (4.5-8.0) and was thermostable up to 60°C. Kinetic analysis revealed a V_{max} of 133.3 U/mg and K_M of 0.9 mg/mL. TLC, HPLC and MALDI-TOF MS analyses confirmed both endo- and exo-acting activities. Synergistic hydrolysis of different xylan substrates by *Ac*Xyn30B_12 and xylobiohydrolase, *Ac*GH30A elevated the TRS production. When used to hydrolyze pretreated biomasses, *Ac*Xyn30B_12 produced both xylo-oligosaccharides and xylose. In-silico structure prediction of 3D model structure of *Ac*Xyn30B_12 using AlphaFold2 and validation by Ramachandran plot and ERRAT analyses demonstrated high model accuracy. Molecular docking of *Ac*Xyn30B_12 with xylotriose (ΔG=−11.2 kcal/mol) confirmed strong substrate affinity. Molecular dynamics simulations of *Ac*Xyn30B_12-xylotriose complex showed reduced RMSD, Rg and SASA values indicating enhanced stability upon ligand binding. SAXS and DLS analyses revealed a monodispersed, globular conformation with a hydrodynamic radius of 4.0 nm. The efficiency of *Ac*Xyn30B_12 was evaluated in enzymatic deinking of waste papers viz. newspaper and notebook paper. The findings establish *Ac*Xyn30B_12 as a robust, multifunctional biocatalyst with promising applications in biomass valorization and sustainable paper deinking industries.