Thermophilic/thermotolerant fungal isolates were isolated from composting soils/ solid industrial waste collected from different regions of India (Jammu and Kashmir, Amritsar and Ahmedabad). The strains were identified morphologically, microscopically and on the basis of rDNA sequence of ITS1-5.8S-ITS2 region were found to produce distinct and multiple endoglucanases and β-glucosidases. The cellulolytic enzyme extracts from these strains were tested for their ability to deink printed composite paper waste. *A. fumigatus* fresenius which produce endoglucanases apparently devoid of cellulose binding module (CBM) resulted in significant deinking of composite paper waste (53%) with increased brightness of 4.32% ISO and improved physical properties of recycled paper sheet. *A. fumigatus* fresenius was taken up further for optimization of production of multicomponent enzymes. Using one factor at a time approach, the culture under optimized conditions produced 238.0, 9.7, 312 and 3792 (U/g DW substrate) of endoglucanase (EG), Fpase, β-glucosidase (β-G) and xylanase, respectively. Further optimization of enzyme production using statistical approach employing Box-Behnken design of experiments was carried out to study the effect of beef extract concentration, tween-80 concentrations and temperature on enzyme production. The R² values computed for endoglucanase, β-glucosidase, Fpase, cellobiohydrolase and xylanase ranged between 97.9% and 99.9%, showing their fitness in predicting the effect of beef extract concentration, tween-80 concentrations and temperature on enzyme production under SSF by *A. fumigatus* fresenius (AMA). It was found that optimal enzyme activities, i.e., 240.2, 9.73, 470, 15 (units/g DW of substrate) of endoglucanase, Fpase, β-glucosidase, and CBH were respectively produced under optimized culture conditions i.e., tween-80 (0.24%), temperature (37°C) and beef extract (0.25%). Using the combination of one factor at a time and RSM optimization approach, 2.43, 2.88 and 1.87 folds increase in the levels of endoglucanase, Fpase, and β-glucosidase activities, respectively, when compared to those observed under unoptimized conditions were achieved. The studies on regulation of expression of endoglucanase, Fpase, β-glucosidase, CBH and xylanase showed that rice straw induced maximal levels of cellulase. Addition of mannitol to rice straw medium resulted in up regulation of EG activity. However, glycerol addition led to repression of two isoforms. The production of β-glucosidases in *A. fumigatus* fresenius was weakly repressed when
fructose, sucrose, glycerol, mannitol and cellobiose was supplemented to medium containing rice straw. The regulation of expression of multiple β-G isoforms was distinctly different when compared to EGs. The sequential expression of multiple isoforms of EGs was found to be influenced by the distinct metabolite footprint profiles comprising of metabolites identified as identified as cellobiose G₂ as well as G₁/G₂ positional isomers in the culture filtrate.

The crude enzyme extract produced under optimized culture conditions was resolved by 2DE. Fifty-seven distinct proteins spots were detected in pI range of 3.0-5.6. The zymograms for detection of β-glucosidase and cellulase (CBHI/EGI) were developed by activity staining using MUG and MUL as substrates, respectively. The activity staining of the gel for β-glucosidases showed the presence of activity in three distinct regions, with a major activity spot (β-G I) appearing at pI~3.2 and molecular weight of ~85 KDa. In addition, five β-glucosidase activity spots were detected; of these, four isoforms of apparently same molecular weight, but of different pI (β-G II), were found as a train of spots and an additional active β-glucosidase spot (β-G III) with pI~5.6 was observed. The resolution of crude extract on single dimension pI gel indicated the presence of four β-glucosidases. For localizing CBHI/EGI zymogram was developed using 4-methylumbelliferyl β-D-lactopyranoside (MUL). All five activity spots for CBHI/EGI activity were detected. This is the first report on localizing CBHI/EGI active spots in 2DE gels. Since MUL is not a specific substrate for assay of CBH I activity, therefore, it could not be ascertained whether the observed spots were of EGI or CBHI. However, by simultaneously developing two zymograms against the proteins resolved on IEF gels (pI 3-10) for CBHI/EGI and endoglucanases using MUL and CMC as respective substrates, we found that only one of the three bands, i.e., CBHI/EGI corresponds to CBHI while other two were apparently EGI. The secretome characterization using peptide mass fingerprinting (LC/MS/MS) of the spots showed presence of combination of variety of glycosyl hydrolases (GH-3, GH-7, GH-12, GH-20, GH-28, GH-43, GH-47, GH-62) in the crude extract of *A. fumigatus* which distinctively differs from the glycosyl hydrolases present in the secretome of cellulase producing industrial strain *Trichoderma reesei*.

The three components of cellulase enzyme (EG, CBH, β-G) were purified from a crude cellulase preparation of *Aspergillus fumigatus* fresenius (AMA) by consecutive column chromatography. The purified enzymes EG, CBH & β-glucosidase were acidic
proteins with pI of 2.5, 4.6 and 3.0 with respective molecular weights (M_r) of 38, 66 and 85 KDa, as estimated by SDS-PAGE. EG exhibit optimal activity at 50°C whereas, CBH and β-glucosidase were optimally active at temperature 60°C. The optimal pH for activity of the EG, CBH and β-glucosidase were 4.0, 5.0 and 9.0, respectively. The presence of DDT, Mercaptoethanol, EDTA, SDS and metal ions such as Cu^{2+}, Mg^{2+}, Mn^{2+}, Zn^{2+} and Na^{+} positively influenced the activity of endoglucanases and β-glucosidase, but the activity was inhibited in the presence of Fe^{2+} and Ca^{2+}. Whereas, increased CBH activity in presence of Fe^{2+} and β-glucosidase activity in presence of Ca^{2+} was observed. Endoglucanase preferentially recognized xyloglucan and barley-β-glucan when compared with carboxy methyl cellulose (CMC) as substrate. K_m and V_max for hydrolysis of xyloglucan (1.3 mM & 3.1 x 10^{2} µmol^{-1}mg protein^{-1}), (1.9 mM & 4.4 x 10^{2} µmol^{-1}mg proteins^{-1}) and CMC (8.3 mM & 0.52 x 10^{2} µmol^{-1}mg protein^{-1}) were observed. Whereas, K_m and V_max for hydrolysis of pNPG by β-glucosidase was calculated as 0.191 x 10^{3} mM and 0.98 x 10^{3} µmol^{-1}mg proteins^{-1}, respectively. Purified β-glucosidase also showed putative transglycosylation activity that was positively catalyzed in presence of methanol as an acceptor molecule.

Saccharification of alkali-treated rice straw with the crude enzyme extract of A. fumigatus fresenius at loading rate of 7, 10 and 15% (w/v) was found to be 84.2, 85 and 46.3%, respectively. When surfactant (0.1%) produced by Trichoderma asperllum was added, the saccharification efficiency improved to 90.0 and 58.8% at loading rate of 10 and 15% (w/v) respectively. The fermentation of the hydrolysate with cocultures of S. cerevisiae and P. stipitis was 2, 2.7 and 3.0% v/v, respectively. The level of ethanol improved to 3.0 and 3.5% v/v in the hydrolysate with 10 and 15% substrate loading rate in presence of surfactant. The HPLC profile of the hydrolysis products obtained by saccharification of alkali pretreated substrate (@ 10% w/v) showed the presence of glucose (550.6 mg), xylose (123.1 mg), xylobiose (60.05 mg), cellobiose (6.83 mg), and cellotriose (6.00 mg) in the resultant hydrolysate, implying that this enzyme mix, though balanced, is deficient in β-xylosidase activity.