SUMMARY & CONCLUSION

The current study elucidates the potential of *Geobacillus thermodenitrificans* X1 (GTX1) for cost-effective xylanase production through valorization of lignocellulosic residues. GTX1 xylanase thus produced was observed as efficient biobleaching agent to be used in paper industry. The major findings have been summarized as follows:

Thermophilic bacteria were isolated from Tattapni hot spring soil sample. All the isolates were screened for xylanase activity through Congo red assay and estimation of xylanase activity in liquid production medium through dinitrosalicylic acid method. The isolate with maximum xylanase activity was identified as *Geobacillus thermodenitrificans* X1, through phylogenetic analysis. Xylanase from GTX1 was produced under submerged fermentation conditions. Various influential physico-chemical and nutritional parameters were optimized one by one for xylanase production, using OFAT method. The optimized conditions attained from OFAT were 1.5% inoculums size, 96 h incubation time, 50°C, pH 8, 1.5% xylan concentration, 0.05g/L of peptone and 200 rpm agitation speed rate. For cost-effective xylanase production, commercial xylan was replaced by cheaper lignocellulosic residues such as wheat straw, rice straw, corn cob and wheat bran. Maximum xylanase production was observed on using wheat straw as substrate. Further, statistical optimization was practiced through RSM to study mutual interactions among the significant parameters which were temperature, agitation speed rate and incubation time. The central composite design of RSM generated 20 different combinations of the selected parameters. Xylanase production was carried out under all the combinations and xylanase activity observed was recorded as the response value for each combination. From ANOVA it was concluded that only one interaction was significant, i.e. between temperature and incubation time. Eventually, the optimized conditions predicted by the statistical software of RSM (Design Expert 10) were 50°C, 87 h and 177 rpm with predicted activity of 23.9 U/mL. Xylanase production was thus carried out under these predicted conditions and 24±2 U/mL activity was observed. The statistical model was thus validated as the experimental value of xylanase activity was close to the predicted one. The crude xylanase produced under optimized conditions was also assayed for accessory cellulases and hemicellulases. Negligible traces of accessory hemicellulases and cellulases were observed; showing endoxylanase is the major enzyme in the crude preparation and is cellulase free. Further, characteristic features of GTX1 xylanase were

studied. The temperature and pH optima was 70°C and 8 respectively and the enzyme showed appreciable thermostability with 90.8% retained activity at 60°C, after 4 h. Also, GTX1 xylanase showed higher stability in alkaline range of pH 8-10. In the presence of Ca^{+2} ions, GTX1 xylanase activity was highly stimulated while it was strongly inhibited by the effect of Mn^{+2} ions. Hydrolytic efficiency of GTX1 was assessed by quantifying the hydrolysis products through HPLC. Variety of xylooligosaccharides were generated; 0.22 ± 0.01 g/L xylose, 1.46 ± 0.02 g/L xylobiose, 1.64 ± 0.02 g/L xylotriose and 0.65 ± 0.01 g/L xylotetrose. Molecular characterization and zymogram of GTX1 xylanase revealed gene lenghth of 1,224 bp (accession no. MG874777) and 45 kDa molecular weight of GTX1 xylanase. Furhter, to enhance the industrial applicability of GTX1 xylanase, it was immobilized through carrier free immobilization to form crude xylanase CLEAs. Conditions for preparation of Xy-CLEAs were optimized and were observed as 1.7 mg/mL of protein concentration, 120 min precipitation time, 0.01% v/v glutaraldehyde as cross-linker and 2 h reaction time for cross-linking. GTX1 Xy-CLEAs prepared were found spherical in shape with 202 nm diameter. Variations in the FTIR spectra of free and immobilized xylanase confirmed structural and functional variations after the preparation of Xy-CLEAs. The optimum pH and temperature of free xylanase and Xy-ClEAs was similar (pH 8 & 70°C), but deviation in the overall pH and temperature profile was observed after immobilization due to conformational alterations. Thermostability of Xy-CLEAs was higher than the free xylanase at both 60°C and 70°C. Tremendous increase in thermostability was observed at 70°C, where retained xylanase activity increased from 15% (free xylanase) to 53% (Xy-CLEAs). Kinetic studies revealed that $t^{1/2}$ value was increased by 69.7% at 60°C and 62.5% at 70°C, after the formation of Xy-CLEAs. Similarly, pH stability was also increased after immobilization; specifically the percent increase was more prominent in the alkaline range. GTX1 xylanase CLEAs also rendered 53.5% reusability after six cycles and retained 86% activity after 8 weeks storage at 4°C. Eventually, the main objective of our study was to estimate the potential of GTX1 xylanase in paper pulp bleaching. Application study was carried out in R&D lab of Kauntum Papers Ltd. paper industry, Hoshiarpur, Punjab. Application of GTX1 xylanase increased the brightness of the paper by 2.2 ISO. Moreover, despite 20% reduction in chlorine dosage, brightness was increased by 1.2 ISO. Paper properties like breaking lenghth and tear factor were tremendously increase after xylanase application; whereas other properties like basis weight, bulk weight, burst factor, viscosity and freeness were comparable with the control.

In nutshell, it could be concluded that thermophilic bacteria *Geobacillus thermodenitrificans* X1 can substantially produce xylanase by utilizing cheaper lignocellulosic substrates. The characteristics of GTX1 xylanase are suitable for its application in the robust conditions of paper industry. Application studies conducted in the present study reinforce the potential of GTX1 xylanase as an efficient biobleaching agent for increasing the brightness and properties of paper. Reduction in the chlorine consumption by application of GTX1 xylanase is an added advantage for curbing the environmental impact.