Summary and Conclusion

Xylanases are hydrolases group of enzymes and due to their depolymerisation activity they have great importance in several industries such as in the paper pulp bleaching industries. Several microorganisms including fungi and bacteria have been reported to readily hydrolyze xylans by expressing 1,4-β-D endoxylanases (EC 3.2.1.8) and β-xylosidases (EC 3.2.1.37). Bacteria, just like in the case of many industrial enzymes, fascinated the researchers for alkaline thermostable xylanase producing trait. Noteworthy members producing high levels of xylanase activity at alkaline pH and high temperature are *Bacillus* spp. The importance of xylanases has not been limited to the paper and pulp industry. Potential applications of xylanases also include bioconversion of lignocellulosic material and agro-wastes to fermentative products, clarification of juices, improvement in consistency of beer and the digestibility of animal feed stock. The enzyme can also be applied for the biosynthesis of xylitol, a polyol alcohol that is used as a sweetener with low calorific value. Since the biotechnological applications require large amounts of low cost enzymes, one of the appropriate approaches for this purpose is to utilize the potential of lignocellulosic wastes.

In this current study two potent xylanase producing microorganism (*P. citrinum* xym2 and *B. subtilis* xym4) have been isolated from the environmental samples. Both the fungal and bacterial isolates were found to produce substantial amount of enzyme using wheat bran as low cost substrates. Moreover, in this research a xylitol producing bacteria, *E. coli* xyl6 was successfully isolated. The research concluded with the following out comes.

1. Xylanase production by the *P. citrinum* xym2 and *B. subtilis* xym4 was optimized to 4 fold and 12 fold, respectively, using wheat bran as raw materials through OFAT and RSM approach.
2. Xylanase from both the sources showed activity in the pH range 3-9 and were thermostable, retaining 80 % activity in the temperature range of 4-60 ^{o}C.
3. *B. subtilis* xym4 was found to be superior than *P. citrinum* xym2 in terms of saccharification and release of xylose from agro residues.
4. Xylanase from *B. subtilis* xym4 was found to have molecular weight of 42 kDa and showed higher catalytic efficiency (Kcat/Km= 11.05).
5. Acid pretreated wheat bran was proved to be the best agro residual substrate that liberated higher quantity of reducing sugars upon XEC^{P} and XEC^{B} treatments.

6. Saccharified broths were used for fermentation by *E. coli* xyl6. XEC^{B} treated wheat bran produced higher level of xylitol in comparison to that treated with $XEC^{P.}$
7. PB and RSM based statistical designs together optimized the process of reducing sugar released by XEC^{B} treatment and fermentation of the extract with *E. coli* xyl6 achieved highest xylitol yield.
8. Among the six different fermentation conditions used in SSF experiments, condition4 showed highest production of xylitol, which was confirmed by GC-MS spectral data.
9. Xylitol production was found to be strongly associated with xylose reductase activity.
10. Low DO and pH facilitated the xylitol production.
11. Higher xylitol concentration (98.4 g/l) and high volumetric productivity of 2.05 g/l/h, under SSF process indicated greater efficiency of the process over sequential statistical optimization method.