CHAPTER 6

Conclusion and Suggestion

Corncob, a low-cost agricultural waste with high xylan content was used as the substrate for production of xylooligosaccharides (XOs). After KOH pretreatment, the recovery yield was 43.69% (w/w). The KOH-treated corncob composed of 65.21% cellulose, 24.67% hemicellulose and 4.29% lignin. The KOH-treated corncob was then subjected to enzymatic hydrolysis by in-house thermostable endo-xylanase from *Streptomyces thermovulgaris* TISTR1948 to produce corncob-XOs. The optimal condition suggested by the central composite design (CCD) was enzyme concentration of 129.43 $U/g_{substrate}$ at 53.80°C and pH 6.17. Under this suggested condition, the XO content was 162.97 $mg/g_{substrate}$ or 752.15 $mg/g_{hemicellulose}$. Moreover, the corncob-XOs were able to enhance the growth of prebiotics lactobacilli of *L. casei* TISTR1463, *L. lactis* TISTR1464 and *L. plantarum* TISTR1465 and were comparable to the commercial XOs.

A crude in-house thermostable endo-xylanase from *S. thermovulgaris* TISTR1948 (xylanase_1948) was purified to 15 folds with a recovery yield of 13.0%. The purified xylanase_1948 had an apparent molecular mass of 46.2 kDa on SDS-PAGE. The purified enzyme was highly stable within a pH range of 4.0–11.5 and was thermostable within a temperature range of 50–70°C. The activity of the enzyme reached a maximum at 65°C. Meanwhile, the half-life was 90 min at 70°C. The activity of enzyme was enhanced by Ca^{2+}, Co^{2+} and Mn^{2+}. In contrast, Hg^{2+}, Pb^{2+} and SDS almost completely inhibited the enzyme activity. The K_m and V_{max} values of the purified xylanase on beech wood xylan were 1.34 mg/mL and 0.072 U/mg (µmol/min/mg), respectively. The purified xylanase was then applied for high-purity XO production from KOH-treated corncob. The results showed that main component of XOs was xylobiose (X2), with very little xylose (X1). An *in vitro* study indicated that the high-purity XOs could enhance the growth of probiotic *L. plantarum* TISTR1465 better than XOs prepared by crude and partially purified xylanases.

The integrated process for XO and bioethanol productions from corncob was investigated. XOs were produced by a consecutive process of KOH treatment and hydrolysis by an in-house thermostable endo-xylanase from *S. thermovulgaris* TISTR1948. XO yield of 22.13 g/L (0.08 $g/g_{raw\ corncob}$) and cellulose-rich corncob (CRC) yield of 0.515 $g/g_{raw\ corncob}$ were obtained. The CRC was further used as the substrate for bioethanol production using a new thermotolerant yeast *Candida glabrata* KY618709 via either the separate hydrolysis and fermentation (SHF) using CRC hydrolysate, or the simultaneous saccharification and fermentation (SSF) using CRC under elevated temperature of 35–42°C. For SHF process, the CRC hydrolysate was firstly produced under the suggested optimal condition from Box–Behnken design of a cellulase concentration of 22.04 FPU/g_{CRC}, 7.8% (w/v) CRC, pH 5.06 and 45.93°C at 150 rpm for 96 h. After 96 h of enzymatic hydrolysis, CRC hydrolysate contained total sugar, glucose, xylose and arabinose of 62.16, 51.21, 10.03 and 0.92 g/L, respectively. The results from SHF showed that strain KY618709 could survive and produce ethanol at 35–42°C. The highest ethanol concentration of 21.92 g/L (0.28 g/g_{CRC}), ethanol productivity of 0.304 g/L/h with 93% theoretical yield was obtained at 40°C. While, SSF of strain KY618709 at 40°C showed the significantly higher ethanol titer (22.35 g/L) than commercial *Saccharomyces cerevisiae*. Moreover, by fed-batch SSF at 11.7% CRC, the ethanol concentration of 31.32 g/L (0.27 g/g_{CRC}), ethanol productivity of 0.33 g/L/h with 89% theoretical yield was achieved.

From this study, it can be concluded that corncob is a potential and inexpensive substrate for a high value chemicals production. The xylan in corncob is converted into XOs using an in-house thermostable endo-xylanase from *S. thermovulgaris* TISTR1948. The results from purification and characterization studies reveal that this purified xylanase has a promising characteristic to produce high-purity XOs which can enhance the growth of probiotic lactobacilli better than that XOs produced from crude xylanase. Besides that, this process not only generates XOs but also CRC. The CRC can be further used as a substrate for bioethanol production via SSF by a new thermotolerant yeast *C. glabrata* KY618709. Moreover, the enzymatic process to produce XOs and bioethanol can be effectively and economically carried out. Hence, this study can be an alternative strategy for a valorization of corncob, a low-cost agricultural waste, into XOs and bioethanol.

Based on the overall results from this study, there are suggestions for further investigation to improve the entire process of XOs and bioethanol production from corncob. According to the pretreatment step, the liquid fraction containing high lignin content might be a potential substrate for high value products such as furfural, lignosulfonate, vanillin and syringaldehyde. Meanwhile, the purification, structural elucidation, prebiotic properties and antioxidant activity of corncob-XOs are also necessary to apply these XOs as the functional food for human consumption. Moreover, the optimization of bioethanol production process to increase the final ethanol concentration should be further investigated.