# CHAPTER 1

# Introduction

## 1.1 Statement and significant of the study

Recently, substantial amounts of global agricultural residues are generated annually for 155 billion tons (Ptasinski, 2015). Disposal of these wastes is regarded as one of global environmental issue (Arevalo-Gallegos et al., 2017). Agricultural residues are attractive source of lignocellulosic materials (LCMs) which can be converted to value-added chemicals according to the biorefinery approach. The products from LCMs conversion includes fine chemicals, animal feed, pulp and paper, biofuels and enzymes (Arevalo-Gallegos et al., 2017). LCMs are promising substrate for biorefinery strategy because they do not have any effects on food production and animal feed unlike starchy and sugar-based feedstock (Limayem and Ricke, 2012). The key bottles neck of biofuels and platform chemicals production from LCMs is a process cost (Maity, 2015). Although, the production cost of products derived from LCMs is higher than the bioconversion of food crops, LCMs is the best choice of substrate for a long term production (Ricardo Soccol et al., 2011). The main components of LCMs are cellulose (45–55%), hemicellulose (25–35%) and lignin (20–30%) which can be hydrolyzed to be the fermentable sugars and further using as the substrate for various type of fine chemical via a microbial bioconversion (Deutschmann and Dekker, 2012).

Corncob is usually considered as the most abundant agricultural residues in Thailand. It contains high content of xylan-type hemicellulose that is suitable for using as a substrate for xylose-based chemicals production (Boonchuay et al., 2014). Among the xylose-based products, xylooligosaccharides (XOs) is a well-known prebiotics which is the nutraceutical that derived from LCMs (Samanta et al., 2015). Two main procedures to produce XOs from several agricultural residues are (1) xylan extraction

process and (2) pretreatment process. In the xylan extraction process, xylan is directly broken down into XOs by a physico-chemical method. Xylan extraction processes includes autohydrolysis (Huang et al., 2016), strong alkali extraction (Murciano Martínez et al., 2016) and thermochemical-acid extraction process (Otieno and Ahring, 2012). These processes require special equipment as well as generate unwanted products such as lignin, monosaccharides and furfural. Moreover, the liquid phase of higher degree of polymerization-XOs (DP-XOs) generated from this process needs to further hydrolyze by enzymatic or acid hydrolysis to obtain lower DP-XOs. Moreover, the XOs purification step is also necessary (Carvalho et al., 2013; Samanta et al., 2015). While, the pretreatment process includes mild alkali (Boonchuay et al., 2014) and acid pretreatment (Zhang et al., 2017) combined with acid or enzyme hydrolysis. During pretreatment process, only lignin-xylan matrix in LCMs is broken down and lignin is dissolved in liquid phase (Deutschmann and Dekker, 2012). Then, the remaining xylan in the treated-LCMs is hydrolyzed by endo-xylanase to obtain lower DP-XOs (Boonchuay et al., 2016). The pretreatment of LCMs has been demonstrated to increase the accessibility of enzyme with LCMs and enhance the purity of XOs (Carvalho et al., 2013). The XOs production from KOH-treated corncob, a xylan-rich LCM, not only lower DP-XOs are desirable attained but the solid waste residue or cellulose-rich corncob (CRC) is also generated. CRC contains high cellulose content (75–80%) that might be a promising substrate for bioethanol production.

XOs are recognized as prebiotics oligosaccharides naturally presented in fruits, vegetables, bamboo shoots, honey and milk (Mäkeläinen et al., 2009). However, their concentration in various foods is insufficient for exhibit the prebiotics properties. Since, the conversion of LCMs into XOs is require to improve the well-being of human and livestock (Samanta et al., 2015). Several health benefits of XOs have been reported such as increasing the probiotic population (Boonchuay et al., 2016; Boonchuay et al., 2014; Moure et al., 2006), providing short-chain fatty acids (Hsu et al., 2004), decreasing the number of pathogenic and putrefactive bacteria in human gastrointestinal tract, and antioxidant activity (Gowdhaman and Ponnusami, 2015; Samanta et al., 2012; Veenashri and Muralikrishna, 2011). The advantages of XOs over other prebiotics have been reported; for example, XOs can resist heat up to 100°C and are stable over a wide range of pH 2.5–8.0 (Deutschmann and Dekker, 2012), and the lower dose of XOs are

required for promoting the bifidobacteria population compare to other oligosaccharies (Mussatto and Mancilha, 2007).

LCMs are not only used as the substrate for XOs production (Bian et al., 2013; Boonchuay et al., 2014), but they are also used as the substrate for bioethanol production (Erdei et al., 2012; Singhania et al., 2014). Generally, bioethanol is produced from three sources of substrate including food crops (first generation), lignocellulosic materials (second generation) and algal biomass (third generation) (Binod et al., 2010; Zhang et al., 2010; Zhou et al., 2014). It has been used as gasoline replacement to reduce petroleum using as well as enhance octane number. Nowadays, the substrates for global ethanol production are corn grain and sugarcane (Balat et al., 2008). While, the ethanol production from LCMs is being increasing dues to its advantages over first generation substrate in that it uses a low-cost substrate, generates small amount of greenhouse gases, employs environmental friendly production process and reduces land-use, water, and energy consumption (Balat et al., 2008; Ricardo Soccol et al., 2011).

This study demonstrates the integrated process for XOs and bioethanol production from corncob by enzymatic methods. The overview of this study can be explained as Figure 1.1.

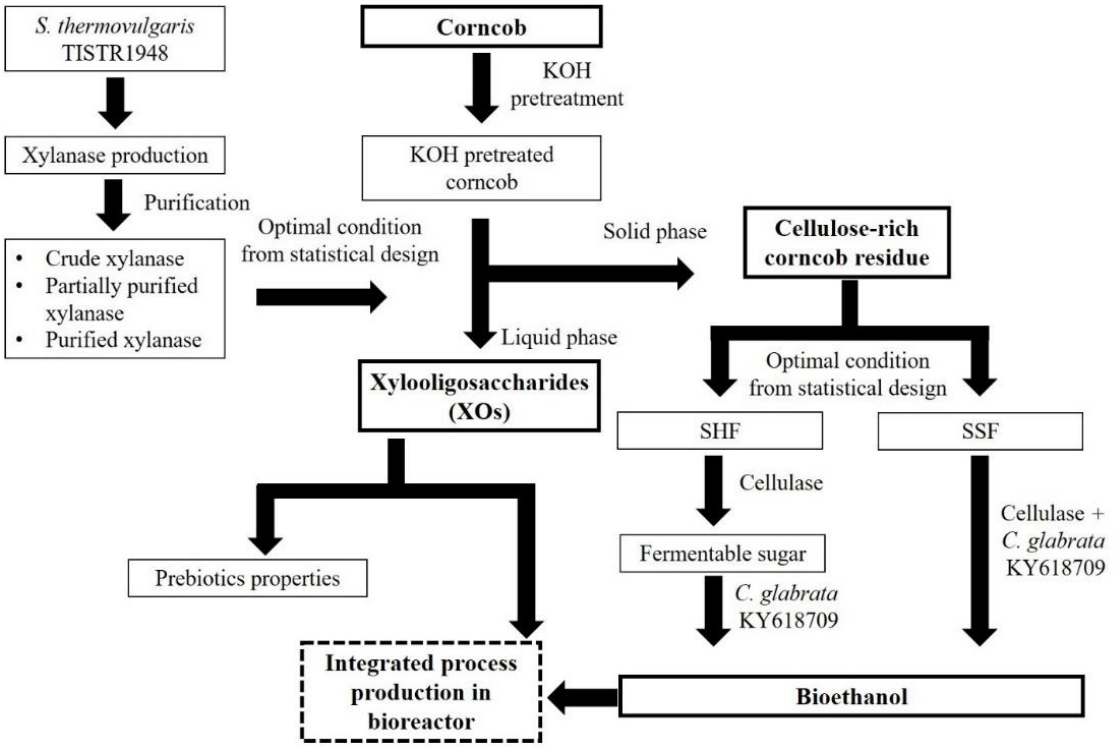


**Figure 1.1** Diagram of the integrated process of xylooligosaccharide and bioethanol production from corncob.

The first step of XO production, corncob has been pretreated by mild alkali (KOH) solution. Then, the treated-corncob has been used as the substrate for XO production by either crude, partially purified or purified xylanases from *Streptomyces thermovulgaris* TISTR1948. The optimal condition for XO production and fermentable sugar production were studied using statistical design via a central composite design and Box–Behnken design (BBD). Moreover, *in vitro* prebiotic properties of corncob-derived XOs were investigated with probiotic lactic acid bacteria. Meanwhile, dried CRC, a solid waste generated from XO production step, has been used as the substrate for bioethanol production via a separate hydrolysis and fermentation (SHF) or simultaneous saccharification and fermentation (SSF) process by a thermotolerant yeast *Candida glabrata* KY618709. XO and bioethanol production in a stirred tank bioreactor is also performed in order to find out the possibility for scaling up of this integrated process.

## 1.2 Objectives

1.2.1 To investigate the integrated process for value adding to corncob, a low cost-agricultural waste by using as the substrate for high valuable products of XOs and bioethanol.

1.2.2 To optimize the XO production from corncob by using statistical design.

1.2.3 To study the purification and characterization of in-house thermostable endo-xylanase from *S. thermovulgaris* TISTR1948, and its application on corncob-XOs production.

1.2.4 To examine the *in vitro* prebiotic properties of high-purity XOs prepared by crude, partially purified and purified xylanases from *S. thermovulgaris* TISTR1948.

1.2.5 To optimize the fermentable sugar production from cellulose-rich corncob (CRC).

1.2.6 To find out the suitable ethanol fermentation process from CRC by using a thermotolerant yeast *C. glabrata* KY618709.

1.2.7 To study the up-scaled production of XOs and bioethanol in the bioreactor.