CHAPTER 5

An Integrated Process for Xylooligosaccharide and Bioethanol Production from Corncob

5.1 Introduction

Nowadays, conversion of lignocellulosic materials (LCMs) to ethanol and value-added chemicals is still a challenging proposition (Menon and Rao, 2012; Singhania, 2009). LCMs are an attractive renewable substrate for bioethanol production that do not have any effect on food production and animal feed (Limayem and Ricke, 2012). Although the conversion cost of cellulosic ethanol is higher than that of other food crops, cellulosic ethanol is the best candidate for long-term production. Ethanol production from LCMs has advantages over first-generation biofuel in that it uses a low-cost substrate, generates a small amount of greenhouse gas, employs an environmentally friendly production process and reduces land use (Ricardo Soccol et al., 2011). Corncob is usually regarded as the most abundant LCM in Thailand. It is xylan-rich LCMs which are suitable for use as a substrate for the production of xylose-based products, especially xylitol and xylooligosaccharides (XOs) (Deutschmann and Dekker, 2012; Egüés et al., 2014). Previous studies have reported a process for XO production from KOH-treated corncobs using both crude and purified in-house thermostable endo-xylanase from *Streptomyces thermovulgaris* TISTR1948 (Boonchuay et al., 2016; Boonchuay et al., 2014). In that process, not only were XOs with a lower degree of polymerization (DP 2−5) desirably obtained, but the solid waste residues, named cellulose-rich corncob (CRC), was also generated. The CRC reached a cellulose content in the range of 78−83% (w/w), which might make it a promising substrate for bioethanol production.

In this study, a new biorefinery strategy is demonstrated for an integrated process for XO production from corncob by using an in-house thermostable endo-xylanase from

S. thermovulgaris TISTR1948, combined with bioethanol production using a new thermotolerant yeast *Candida glabrata* KY618709. This integrated process which involves the whole process for XO and bioethanol production may also be useful for increasing the efficiency of these high-value products as well as lowering the total cost of production.

5.2 Materials and Methods

5.2.1 Chemicals and materials

Corncob and rice straw were kindly donated by local farmers in Chiang Mai and Phayao provinces, Thailand, in January of 2016. Corncob was used as the substrate for an integrated process to produce XOs and bioethanol. Rice straw was used as a carbon source for an in-house thermostable endo-xylanase production. Loog-Paeng, a traditional alcoholic beverage starter culture, was purchased from a local market in Khon Kaen province, Thailand. The substrate for fermentable sugar production was CRC, a solid waste from the XO production process.

A commercial cellulase cocktail (iKnowZyMe AC cellulase) was purchased from Reach Biotechnology (Bangkok, Thailand). The endo-glucanases, FPase, β-glucosidase and xylanase were used at concentrations of 1200, 50, 140 and 2,100 U/mL, respectively. Beech wood xylan, *p*-nitrophenyl-β-D-glucopyranoside (*p*NPG), xylose (X1), arabinose, glucose, dinitrosalicylic acid (DNS) and cellobiose were purchased from Sigma (St. Louis, MO, USA). Xylobiose (X2), xylotriose (X3), xylotetraose (X4) and xylopentaose (X5) were purchased from Megazyme (Wicklow, Ireland). Other chemicals used in this study were of analytical grade.

5.2.2 Analysis of xylooligosaccharides, fermentable sugar and ethanol

Aliquot samples were filtered through a nylon membrane filter (0.2 μm, FiltrEX, USA) and subjected to HPLC analysis (SCL-10Avp; Shimadzu, Kyoto, Japan) with an Aminex HPX 87H column (300×7.8 mm; Bio-Rad, Hercules, USA). The mobile phase consisted of 5.0 mM H_2SO_4 as an eluent at a flow rate of 0.45 mL/min for xylanase-hydrolyzed sugars (Boonchuay et al., 2014) and 0.60 mL/min for cellulase-hydrolyzed sugars and ethanol (Aguiar et al., 2005; Qureshi et al., 2015). The column thermostat was

set at 40°C. Sugar and ethanol were detected using RI detector (refractive index detector RID-10A) in a linear gradient over 25 min (Appendix A-2 and A-3).

5.2.3 Enzyme assays

The endo-glucanase activity assay was modified from the method of Zhang et al. (2009), using 0.5% (w/v) sodium carboxymethyl cellulose (Na-CMC) solution in 0.1 M sodium-citrate buffer (pH 5.0) as a substrate. The reaction was carried out at 50°C for 10 min. Release of reducing sugars was measured using the DNS method. One unit of endo-glucanase activity (U) was defined as the amount of enzyme liberating 1.0 μmol of reducing sugar (as glucose) per min (Appendix A-5).

Total cellulase activity (FPase) was measured using filter paper as substrate, according to the modified method of Ghose (1987). Briefly, enzyme was mixed with 1.0 mL of 0.1 M sodium-citrate buffer (pH 5.0) and incubated at 50°C with 50 mg of Whatman No. 1 filter paper strip (0.1 × 0.6 cm) for 60 min. Release of reducing sugars was measured using the DNS method (Miller, 1959). One unit of FPase activity (U) was defined as the amount of enzyme liberating 1.0 μmol of reducing sugar (as glucose) per min under the assay conditions (Ghose, 1987) (Appendix A-6).

β-Glucosidase activity was measured using *p*-nitrophenyl-β-D-glucopyranoside *p*NPG as substrate. Briefly, 5 mM *p*NPG in 0.1 M sodium-citrate buffer pH 5.0 (0.9 mL) was mixed with 0.1 mL of enzyme and incubated for 10 min at 50°C. The reaction was stopped by the addition of 0.1 mL saturated sodium tetraborate solution (Salma, 2008). One unit (U) of enzyme was defined as the amount of enzyme liberating 1.0 μmol *p*-nitrophenol/mL/min under the conditions determined. The concentration of *p*-nitrophenol released was calculated using the molar extinction coefficient, ε 400 = 18,300 M^{-1} cm^{-1} (Salma, 2008) (Appendix A-7).

Xylanase activity was measured using 1.0% (w/v) beech wood xylan solution in 0.1 M potassium-phosphate buffer (pH 6.5) as substrate. The clear supernatant was diluted in 0.1 M potassium-phosphate buffer (pH 6.5) and incubated at 55°C with beech wood xylan solution for 10 min. Release of reducing sugars was measured using the DNS method. One unit (U) of xylanase activity was defined as the amount of enzyme liberating 1.0 μmol of

reducing sugar (as xylose) per min under assay conditions (Chaiyaso et al., 2011) (Appendix A-4).

5.2.4 Characterization of cellulose-rich corncob and mass balance of the process

The cellulose (TAPPI T-203-cm-99), hemicellulose (TAPPI T-203-cm-99) and lignin (TAPPI T-222-om-02) content of those materials were determined by the TAPPI method which was analyzed by the Animal Nutrition Laboratory, Department of Animal and Aquatic Sciences, Faculty of Agriculture, Chiang Mai University (Romaní et al., 2012). The mass balance of the integrated process was calculated according to the composition of cellulose and hemicellulose in corncob, as well as the products derived from those compositions. Dried samples of each step were mounted on stubs, placed on conductive carbon tape and coated with gold using a sputter coater (JFC-1200, JEOL) at 15 mA for 150 s. Then, gold-coated samples were viewed under a scanning electron microscope (SEM; JEOL 5410-LV, JEOL, Japan) to observe the morphology, surface area and physical structure.

5.2.5 Microorganisms and culture conditions

S. thermovulgaris TISTR1948 was used as an in-house thermostable endo-xylanase producer and its crude enzyme was used for XO production, as described previously (Chaiyaso et al., 2011).

Thermotolerant yeast was isolated from a traditional alcoholic beverage starter culture (Loog-Paeng). Ethanol-producing thermotolerant yeasts were selected according to their growth performance and ability to produce ethanol at 37–42°C. The selected yeast strains were identified based on their 26S rDNA gene sequence which was analyzed by the Mahidol University and Osaka University Collaborative Research Center of Bioscience and Biotechnology (MU-OU: CRC), Mahidol University, Thailand. The 26S rDNA sequences accessible in GenBank by a BLAST search of the National Center Biotechnology Information (NCBI) databases (Appendix C). The promising thermotolerant and bioethanol-producing yeast strain was identified as *C. glabrata* with 100% identity and deposited in GenBank with Accession number KY618709. Commercial active dry yeast (*Saccharomyces cerevisiae*) was purchased from Danstil Inc. (Denmark). An inoculum for

ethanol fermentation was prepared in yeast malt medium (Appendix B-1). Yeast strains were grown at 37°C and 200 rpm in a shaking incubator (LSI-3016R, Labtech, Korea). After 24 h, the 10% (v/v) inoculum (OD_{600} = 6.00) was inoculated to the fermentation medium.

5.2.6 Integrated process for xylooligosaccharide and bioethanol production

An overview of the integrated process to produce XOs and bioethanol from corncob is shown in Figure 5.1.

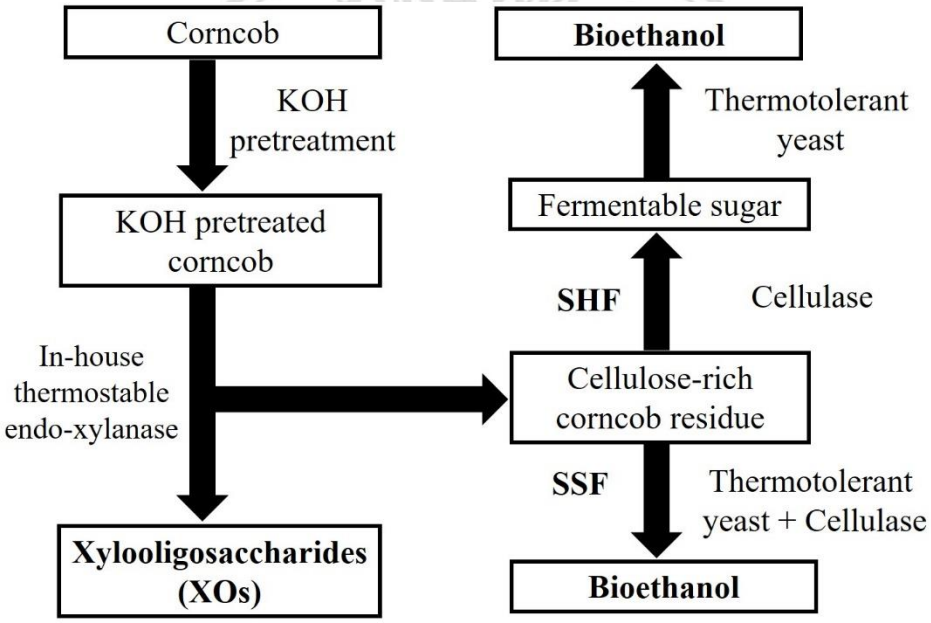

Figure 5.1 Schematic process of xylooligosaccharide and bioethanol production from corncob.

5.2.6.1 First step of xylooligosaccharide production in a 5-L stirred tank bioreactor

i) Optimization of KOH pretreatment of corncob was conducted in a 10-L stainless steel reactor. Briefly, dried corncob powder was prepared using a hammer mill and 100 mesh sieving. The corncob (10% (w/v)) was treated with various KOH concentrations of 2.5, 5, 10, 15 and 20% (w/v) at 90°C for 1 h. Then, the pH of sample was adjusted to the neutral at pH 7.0 by adding of H_2SO_4. After that, the solid fractions were recovered by filtering through the muslin cloth and washing with tap water. These solid fractions were

dried at 80°C in an oven (UN110; Memmert, Germany) until the constant weight was obtained. These KOH-treated corncob samples were subjected to produce XOs by in-house thermostable endo-xylanase according to the method of Boonchuay et al. (2014).

ii) The XO production was performed in a 5-L stirred tank bioreactor (MDFT-N-5L, BE Marubishi, Bangkok, Thailand) with a reaction volume of 3.5 L. The experiment conditions were simplified from the laboratory scale, according to the report (Boonchuay et al., 2014). Briefly, the KOH-treated corncob was used as a substrate for XO production and performed at a solid loading of 15% (w/v) in potassium-phosphate buffer pH 6.2. An in-house thermostable endo-xylanase from *S. thermovulgaris* TISTR1948 was used to hydrolyze KOH-treated corncob. The hydrolysis procedure was operated at 54°C for 12 h. After that, the reaction mixture was separated by filtering through filter paper (Whatman No. 4) into liquid fraction (XOs) and solid fraction (CRC). XOs in the liquid phase were analyzed by HPLC. The clear liquid phase of XOs was demineralized and subjected to spray-drying to obtain XO powder (Boonchuay et al., 2014).

5.2.6.2 Second step of bioethanol production

The solid phase of CRC was separated, washed with tap water and dried at 80°C until constant weight was obtained and kept at 4°C for further experiments. Dried CRC was used as the substrate for bioethanol production via separate hydrolysis and fermentation (SHF) or simultaneous saccharification and fermentation (SSF) with thermotolerant yeast strain KY618709.

i) A second order Box–Behnken design (BBD) was employed for the statistical optimization of condition for fermentable sugar production from CRC via a response surface methodology (RSM). The Design Expert 6.0.10 software (Stat-Ease, Minneapolis, MN, USA) was used to design and analyze the experiments and generate the response surface graphs. Twenty-nine experiments that generated from BBD were performed to test the effect of four independent variables at three different levels; enzyme concentration (X_1, 20–60 FPU/g_{CRC}), pH (X_2, 3.5–6.5), temperature (X_3, 35–65°C) and CRC concentration (X_4, 2.5–17.5% (w/v)) (Table 5.1). The samples from each experiment were taken after 96 h hydrolysis time and analyzed by HPLC. The data obtained from the experiments were

analyzed and tested for the validation of the model in both of a flask-scale with a 100 mL reaction volume and a bioreactor-scale with a 3.5 L reaction volume.

Table 5.1 Experimental codes, ranges and levels of independent variables in the response surface methodology experiment.

Variables	**Units**	**Symbol codes**	**Levels**		
			Low (-1)	**Center (0)**	**High (+1)**
Enzyme concentration	FPU/g_{CRC}	X_1	20.0	40.0	60.0
pH	-	X_2	3.5	5.0	6.5
Temperature	°C	X_3	35.0	50.0	65.0
CRC concentration	% (w/v)	X_4	2.5	10.0	17.5

ii) For the SHF process, CRC hydrolysate was produced using the optimal conditions from a BBD with a cellulase concentration of 22.04 FPU/g_{CRC}, 7.8% (w/v) CRC, pH 5.06 and 45.93°C at 150 rpm. After 4 days of hydrolysis, the whole CRC hydrolysate was used as substrate (fermentable sugar) for bioethanol production by thermotolerant *C. glabrata* KY618709 and *S. cerevisiae* was used as the control for ethanol production. The fermentation medium consisted of CRC hydrolysate (51.21±1.43 g/L glucose), 4.0 g/L $(NH_4)_2SO_4$, 1.0 g/L yeast extract, 1.0 g/L $NH_4H_2PO_4$, 0.1 g/L $MgSO_4.7H_2O$ and 200 ppm $K_2S_2O_5$ and was incubated at 37°C for 24 h (Appendix B-4). The medium was then inoculated with 10% by volume of KY618709 or *S. cerevisiae*. The effect of temperature on bioethanol production of the two yeast strains was carried out at 35, 37, 40 and 42°C in 100-mL laboratory bottles (Duran, Germany) equipped with an airlock and a working volume of 90 mL, for 5 days. Samples were taken at 0, 6, 12, 24, 36, 48, 72 and 120 h and analyzed by HPLC.

iii) For the SSF process, 7.8% (w/v) of CRC was supplemented with 4.0 g/L $(NH_4)_2SO_4$, 1.0 g/L yeast extract, 1.0 g/L $NH_4H_2PO_4$ and 0.1 g/L $MgSO_4.7H_2O$ and dissolved in sodium-citrate buffer pH 5.06. The SSF fermentation medium was autoclaved at 121°C for 20 min (Appendix B-4). After that, SSF was started by adding 10% (v/v) of a pre-culture solution of KY618709 or *S. cerevisiae*. The effect of temperature on bioethanol production was determined as described in section (ii) and the same sampling method was used.

iv) The bioethanol production by thermotolerant yeast in a bioreactor via SSF was performed using CRC as substrate in a 5-L stirred tank bioreactor with a reaction volume of 3.5 L. SSF experiments were conducted at three conditions: batch SSF with 7.8% (w/v) CRC, fed-batch SSF with 11.7% (w/v) CRC and fed-batch SSF with 15.6% (w/v) CRC in sodium-citrate buffer pH 5.06 and supplemented with nutrients as previously described. The medium was autoclaved at 121°C for 30 min before inoculation. The batch experiment was performed in the bioreactor with 7.8% (w/v) solid initial loading, at 150 rpm, pH 5.06, inoculum size 10% (v/v) and enzyme loading 22.04 FPU/g_{CRC} at 40°C for 5 days under limited oxygen conditions.

A fed-batch experiment with 11.7% (w/v) CRC was conducted to increase the solid loading from 7.8% to 11.7% (w/v) by keeping other parameters the same as for batch SSF. The fed-batch experiment with 11.7% (w/v) CRC was initiated with 7.8% (w/v) solid loading. Then, 3.9% (w/v) sterile CRC was fed at 36 h to increase the substrate loading to 11.7% (w/v). Meanwhile, the fed-batch experiment with 15.6% (w/v) CRC was initiated with 7.8% (w/v) solid loading. Then 3.9% (w/v) CRC was fed at both 36 and 72 h, to increase the final substrate loading to 15.6% (w/v). Samples were periodically taken, as previously described for SHF, for HPLC analysis.

5.2.6.3 Calculation and statistical analysis

All experiments were carried out as triplicate samples. The data were analyzed for statistical significance using one-way analysis of variance (ANOVA) followed by Duncan's multiple range test (p<0.05). The statistical software package SPSS v.17 was used in the analysis of the experimental data.

The XO yield was calculated according to the following equation:

$$\text{XOs yield (g/g)} = \frac{\text{XO concentration (g/L)}}{\text{initial concentration of KOH-treated corncob(g/L)}}$$

The fermentable sugar yield was calculated according to the following equation:

$$\text{Fermentable sugar yield (g/g)} = \frac{\text{sugar concentration (g/L)}}{\text{initial concentration of corncob(g/L)}}$$

The conversion of cellulose to glucose in enzymatic hydrolysis, or hydrolysis efficiency, (%) was calculated according to the following equation:

$$\text{Hydrolysis efficiency (\%)} = \frac{\text{glucose concentration (g/L)} \times V \times 0.9}{\text{cellulose content} \times m} \times 100$$

Where, V is the volume of enzymatic hydrolysis (L) and m is the mass of dried CRC (g) (Lu et al., 2012).

Initial glucose in the SSF fermentation medium was calculated by the following equation:

$$\text{Initial glucose (g)} = \text{hydrolysis efficiency (\%)} \times \text{cellulose content in CRC} \times m$$

The theoretical ethanol yield (Y, %) was calculated according to Liu et al. (2015), by the following equation:

$$\text{Y (\%)} = \frac{\text{ethanol produced (g)}}{\text{initial glucose (g)} \times 0.511} \times 100$$

Where 0.511 is the theoretical value of ethanol conversion efficiency (Liu et al., 2015).

Conversion of cellulose to ethanol (%) was calculated according to Lu et al. (2012), using the following equation:

$$\text{Conversion of cellulose to ethanol (\%)} = \frac{\text{ethanol}_1\text{ (g/L)} - \text{ethanol}_0\text{ (g/L)}}{0.511 \times (\text{biomass}_0) \times f \times 1.111} \times 100$$

Where 1.111 is a conversion factor for cellulose to equivalent glucose, Biomass_0 is initial dry biomass concentration, f is the cellulose fraction of dry biomass, 0.511 is the conversion factor for glucose to ethanol based on the stoichiometric biochemistry of yeast, ethanol_1 is final ethanol concentration and ethanol_0 is initial ethanol concentration (Lu et al., 2012).

The ethanol yield (Y_{EtOH}, g/g) was calculated according to Liu et al. (2015), using the following equation:

$$Y_{EtOH}(g/g) = \frac{\text{ethanol concentration (g/L)}}{\text{initial cellulose in CRC (g/L)}}$$

The ethanol production rate (Q_p, g/L/h) was calculated according to the following equation:

$$Q_p\ (g\ L^{-1}\ h^{-1}) = \frac{\text{final ethanol concentration (g/L)} - \text{initial ethanol concentration (g/L)}}{(T - T_0)}$$

where T is the final ethanol fermentation time (h) and T_0 is the initial ethanol fermentation time (h) (Liu et al., 2015).

5.3 Results and Discussion

5.3.1 Optimal condition for KOH pretreatment of corncob

After pretreatment by 2.5, 5, 10, 15 and 20% (w/v) KOH, the treated-corncob recovery yields were 69.12±1.40, 64.11±1.71, 45.55±1.93, 44.34±1.71 and 39.67±0.88% (w/w), respectively (Table 5.2). Generally, the alkali solution results in a saponification reaction of ester bonds between hemicellulose and, lignin or other substitutions. Moreover, the alkali pretreatment gives more advantages than other pretreatment methods because of a less sugar degradation during pretreatment process (Chen et al., 2017). After enzymatic hydrolysis of 2.5, 5, 10, 15 and 20% (w/v) KOH-treated corncob, the total XO yields of 0.094±0.003, 0.113±0.004, 0.086±0.00, 0.075±0.001 and 0.067±0.004 $g/g_{KOH\text{-treated corncob}}$ were achieved. While, the hydrolysis of raw corncob showed only 0.022±0.002 $g/g_{raw\ corncob}$ of total XOs. Therefore, to retrieve the maximum XO yield from treated-corncob, the best pretreatment condition was 5% (w/v) KOH, 90°C for 1 h.

Table 5.2 The effect of KOH concentration on the recovery yield and xylooligosaccharide production from corncob by an in-house thermostable endo-xylanase from *Streptomyces thermovulgaris* TISTR1948.

KOH (% (w/v))	Recovery yields (%)*	Production yields ($g/g_{KOH\text{-}treated\ corncob}$)**						
		X ≥ 5	X4	X3	X2	X1	Ara	Total XOs
Ctrl***	-	0.010±0.001^{d}	0.000±0.000^{d}	0.012±0.001^{a}	0.000±0.000^{e}	0.005±0.000^{d}	0.002±0.000^{c}	0.022±0.002^{f}
2.5	69.12±1.40^{a}	0.019±0.005^{ab}	0.011±0.001^{ab}	0.006±0.001^{bc}	0.060±0.002^{b}	0.012±0.001^{b}	0.015±0.001^{b}	0.094±0.003^{b}
5.0	64.11±1.71^{b}	0.024±0.001^{a}	0.012±0.001^{a}	0.007±0.001^{b}	0.071±0.001^{a}	0.013±0.000^{a}	0.016±0.001^{b}	0.113±0.004^{a}
10.0	45.55±1.93^{c}	0.017±0.002^{bc}	0.010±0.001^{ab}	0.004±0.001^{c}	0.057±0.001^{c}	0.013±0.001^{ab}	0.017±0.000^{a}	0.086±0.004^{c}
15.0	44.34±1.71^{c}	0.012±0.001^{cd}	0.009±0.001^{b}	0.014±0.001^{a}	0.041±0.001^{d}	0.009±0.000^{c}	0.015±0.000^{b}	0.075±0.001^{d}
20.0	39.67±0.88^{d}	0.009±0.001^{d}	0.005±0.001^{c}	0.012±0.001^{a}	0.042±0.001^{d}	0.010±0.000^{c}	0.017±0.001^{a}	0.067±0.004^{e}

Values are presented as mean ± standard deviation, n = 3. Data with the same superscript in the same column are not significantly different at $p \leq 0.05$. The level of significance was tested by Duncan's multiple range test at $p \leq 0.05$.

*Recovery yield: Recovery yield after KOH pretreatment; **Production yield: XOs and other sugars yield from in-house thermostable endo-xylanase (X≥5, xylopentaose and higher DP-XOs; X4, xylotetraose; X3, xylotriose; X2, xylobiose; X1, xylose; Ara, arabinose); ***Ctrl: Raw corncob was used as the substrate for XO production.

5.3.2 Production of xylooligosaccharides in a 5-L stirred tank bioreactor

After 12 h of suitable hydrolysis time for XO production, catalyzed by an in-house thermostable endo-xylanase from *S. thermovulgaris* TISTR1948, which was conducted in a 5-L stirred tank bioreactor, the liquid and solid fractions were separated by filtration. The liquid fraction was further used as XOs from corncob. The corncob-XOs were analyzed for content of XOs, xylose and arabinose. The XO concentration of 22.13 g/L (0.147 $g/g_{KOH\text{-}treated\ corncob}$) or 0.115 $g/g_{raw\ corncob}$ was obtained. The XOs were composed of 52.58% xylobiose (X2), 11.27% xylotriose (X3), 14.57% xylotetraose (X4) and 21.57% xylopentaose (X5) and higher-DP XOs (Figure 5.2). Endo-xylanases are the key enzymes for xylan degradation (Van Dyk and Pletschke, 2012). The in-house thermostable endo-xylanase from *S. thermovulgaris* TISTR1948 was classified as GH10 endo-xylanase, which hydrolyzes corncob xylan into short-chain XOs with a high X2 content (Boonchuay et al., 2016). Moreover, corncob XOs produced from this xylanase showed prebiotic potential by promoting the growth of probiotic lactobacilli (Boonchuay et al., 2016; Boonchuay et al., 2014). In comparison, the commercial XOs (Wako, Japan) contained 31.73% of X2, 4.66% of X3, 15.50% of X4 and 48.11% of X5 and higher-DP XOs (Figure 5.2). In functional food production, X2 is the most favorable product because its fermentation gives higher kinetic values than other XOs (Uçkun Kiran et al., 2013). From these characteristics, the corncob XOs is promising prebiotic for functional food application.

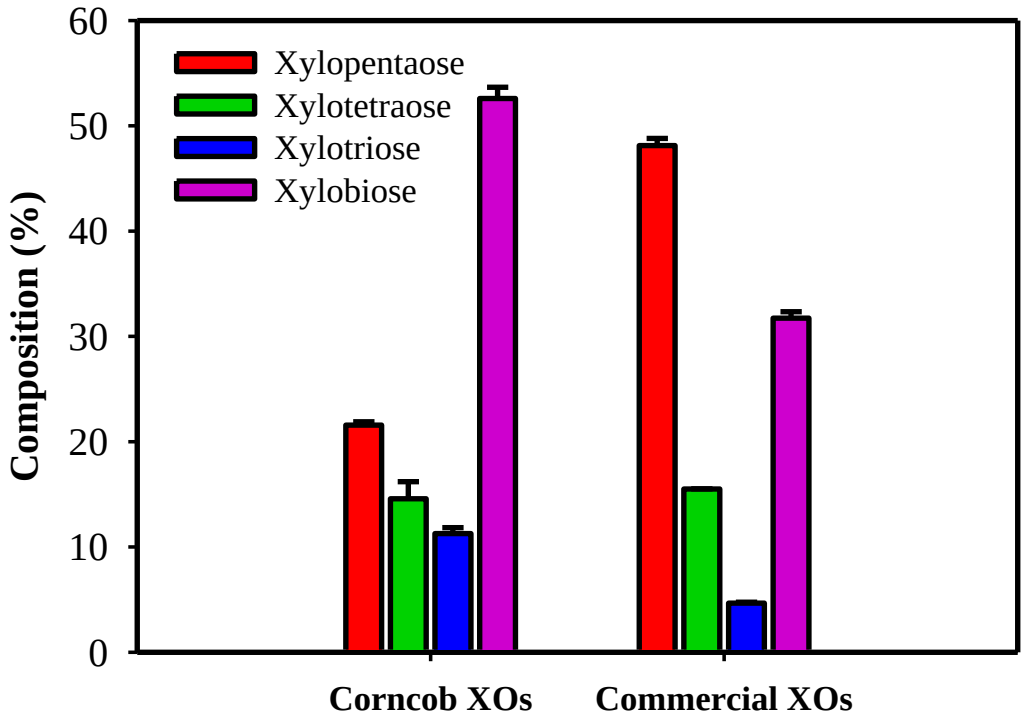

Figure 5.2 Composition of corncob-xylooligosaccharides compared with commercial xylooligosaccharides.

5.3.3 The optimal condition for fermentable sugar production

The data obtained from the BBD experiment were reported as a response of glucose, xylose, arabinose and fermentable sugar and then analyzed by Design expert software (Table 5.3). Analysis of variance (ANOVA) for response surface quadratic model showed that the model of glucose, xylose, arabinose and fermentable sugar were significant with the *p*-value of 0.0001, 0.0121, <0.0001 and <0.0001, respectively (Table 5.4). The coefficient of variation for the model for glucose (R^2 = 0.8980), arabinose (R^2 = 0.9178) and total sugar (R^2 = 0.9145) were represented and they implied a high correlation between the experimentally observed. The significant model for glucose, xylose, arabinose and total sugar were demonstrated by the larger of *F*-test value, smaller of *p*-value and R^2 value closed to 1.00 (Anuar et al., 2013; Bai et al., 2015). Whereas, an R^2 greater than 0.80 is considered as the good and fit model (Xue et al., 2016). So, the R^2 value of xylose model was less than 0.8 indicating non-significant

model. The BBD generated the equation for glucose, xylose, arabinose and total sugar production yield as following equations.

Glucose ($g_{glucose}/g_{CRC}$) = $-2.3440-0.0018X_1+0.3287X_2+0.1033X_3-0.060X_4+0.0001X_1^2-0.0456X_2^2-0.0014X_3^2-0.0004X_4^2-0.0006X_1X_2-0.0001X_1X_3-0.0001X_1X_4+0.0043X_2X_3-0.0031X_2X_4+0.0016X_3X_4$

Xylose (g_{xylose}/g_{CRC}) = $-0.90979-0.00983X_1+0.10891X_2+0.03545X_3-0.02162X_4+0.00003X_1^2-0.01291X_2^2-0.00041X_3^2-0.00017X_4^2-0.00019X_1X_2-0.00003X_1X_3-0.00003X_1X_4+0.00040X_2X_3+0.00053X_2X_4+0.00047X_3X_4$

Arabinose ($g_{arabinose}/g_{CRC}$) = $0.06848-0.00003X_1+0.00733X_2-0.00301X_3-0.00264X_4+0.00001X_1^2-0.00154X_2^2+0.00001X_3^2-0.00003X_4^2-0.00003X_1X_2+0.00002X_1X_3$ $0.00003X_1X_4+0.00013X_2X_3+0.00024X_2X_4+0.00007X_3X_4$

Fermentable sugar (g_{sugar}/g_{CRC}) = $-3.1853-0.0027X_1+0.4449X_2+0.1358X_3-0.0843X_4+0.0001X_1^2-0.0600X_2^2-0.0018X_3^2-0.0006X_4^2-0.0008X_1X_2-0.0001X_1X_3-0.0001X_1X_4+0.0049X_2X_3-0.0023X_2X_4+0.0022X_3X_4$

Even model of arabinose production was significantly, the concentration of arabinose in CRC hydrolysate was very low compared to xylose and glucose. Hence, only model of total sugar was selected for further used in the prediction of fermentable sugar production from CRC. According to Table 5.4, the interaction term between enzyme concentration and pH (X_1X_2) was non-significant term for all responses. On the other hand, the interaction term between temperature and CRC concentration (X_3X_4) was significant term for all responses. While, the interaction term between pH and temperature (X_2X_3) was found to be a significant-term for glucose, arabinose and total sugar production.

Enzyme and substrate loading are crucial factors for the production cost of fermentable sugar from LCMs. Besides that, pretreatment method as well as nature of substrate may also have an effect on enzyme loading (Van Dyk and Pletschke, 2012). In this study, we found that enzyme loading was not significant term (p-value = 0.1279). The cellulase concentration between 20–60 FPU/g_{CRC} did not show a significant effect on glucose and total sugar concentration. It was probably that CRC contained relatively

low lignin content and amorphous structure that providing better accessible substrate for enzymatic hydrolysis (Figure 5.3). Therefore, CRC is promising to be the cost-effectively substrate for fermentable sugar production because high enzyme loading level is not necessary. Another principal factor for enzymatic hydrolysis of lignocellulosic materials is substrate loading.

The results indicated that the glucose, xylose, arabinose and total yields (g/g_{CRC}) increased when decreased the substrate loading. However, at low substrate loading experiment, the glucose and total sugar concentration (g/L) was lower than that of high substrate loading experiment. This might be the unique of CRC structure and the concentration of the initial substrate. However, the high substrate loading may cause the feedback inhibition to the enzyme and increase viscosity of reaction mixture. Therefore, the optimal substrate loading is required for the economically enzymatic hydrolysis process of the LCMs (Van Dyk and Pletschke, 2012).

The response surface graphs for four responses are shown in Figure 5.4–5.7. The response surface analysis suggested the optimal condition for the fermentable sugar production as: enzyme concentration of 22.04 FPU/g_{CRC}, pH 5.06, 45.93°C and 7.8% (w/v) CRC. Validation of the model was performed in both of a flask-scale and a stirred tank bioreactor. Under the suggested condition operating in bioreactor, the fermentable sugar concentration was 62.16±1.03 g/L with glucose 51.21±1.43 g/L, xylose 10.03±0.49 g/L and arabinose 0.92±0.00 g/L. The experimental values were in good agreement with predicted value, which were nearly 7.0% lower than predicted value for total sugar. Therefore, the BBD optimized model was realizable. Moreover, this optimal condition could enhance the total sugar yields by 42% which was higher than un-optimized condition.

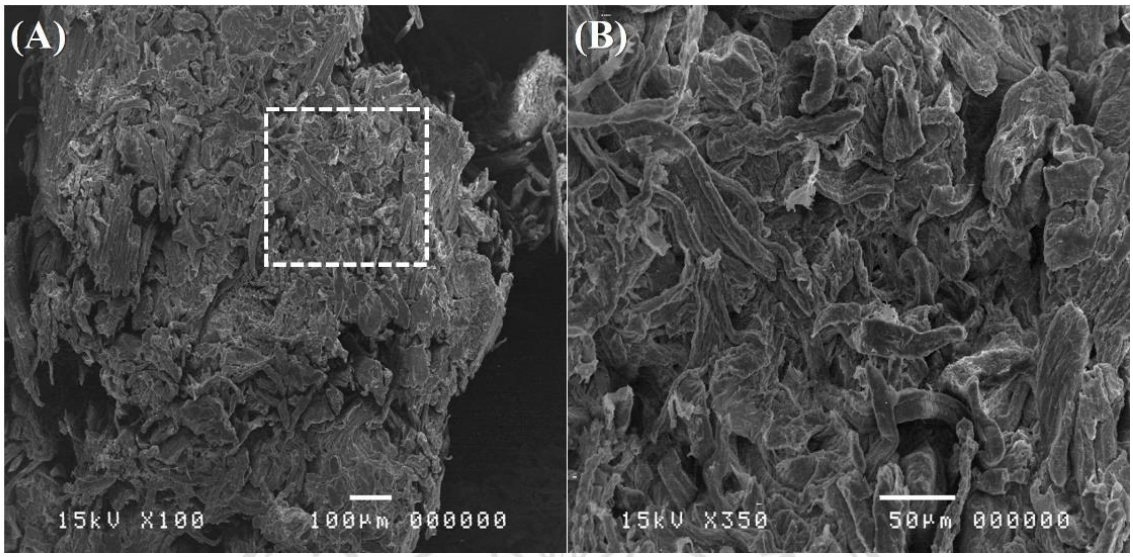

Figure 5.3 Morphological observations of cellulose-rich corncob by scanning electron microscope. The dashed lines in (A) 100× demonstrate the scanning area corresponding to (B) 350×.

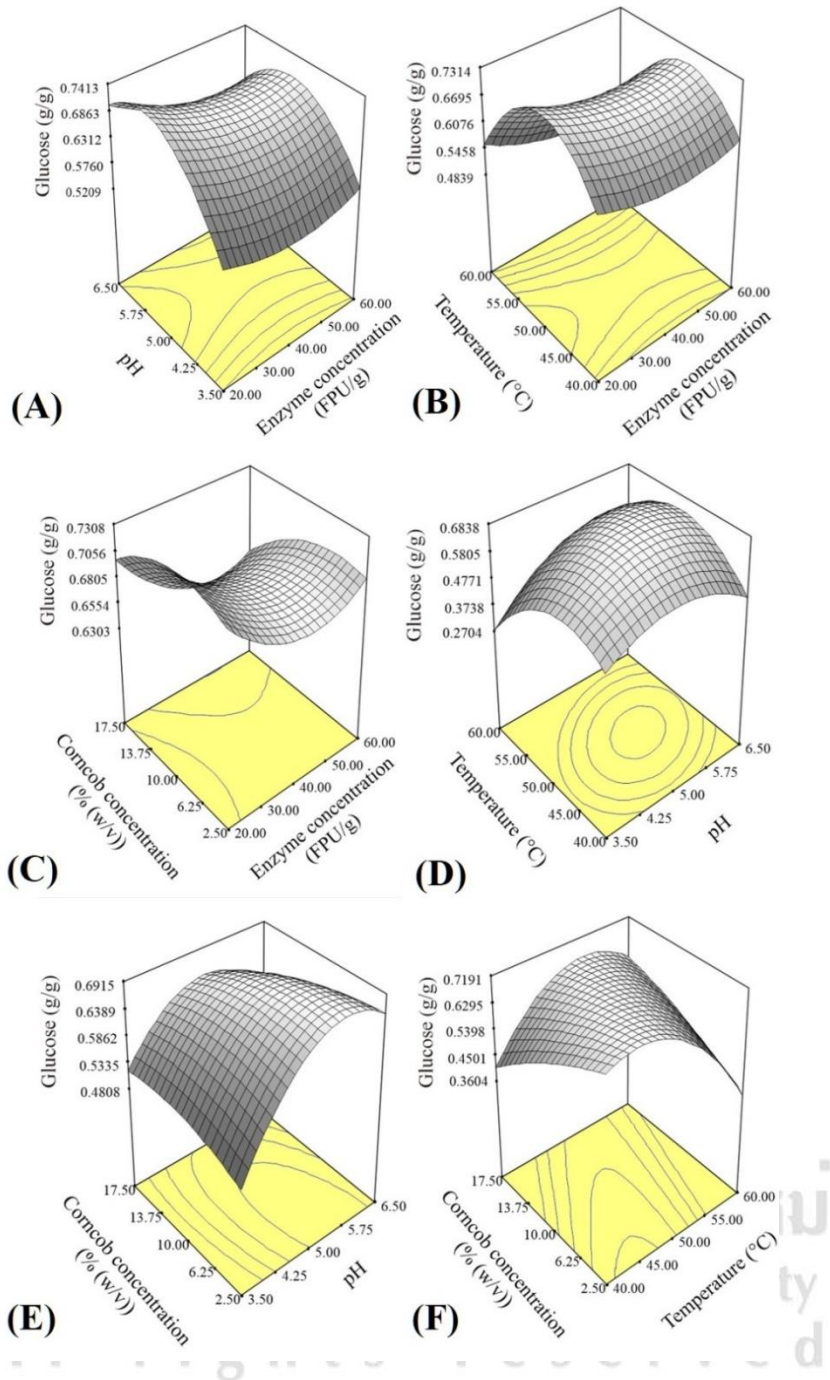

Figure 5.4 Glucose production from cellulose-rich corncob in three-dimensional graphic for quadratic response surface optimization. The comparison was made between (A) enzyme concentration and pH value (X_1X_2), (B) enzyme concentration and temperature (X_1X_3), (C) enzyme concentration and cellulose-rich corncob concentration (X_1X_4), (D) pH value and temperature (X_2X_3), (E) pH and cellulose-rich corncob concentration (X_2X_4) and (F) temperature and cellulose-rich corncob concentration (X_3X_4).

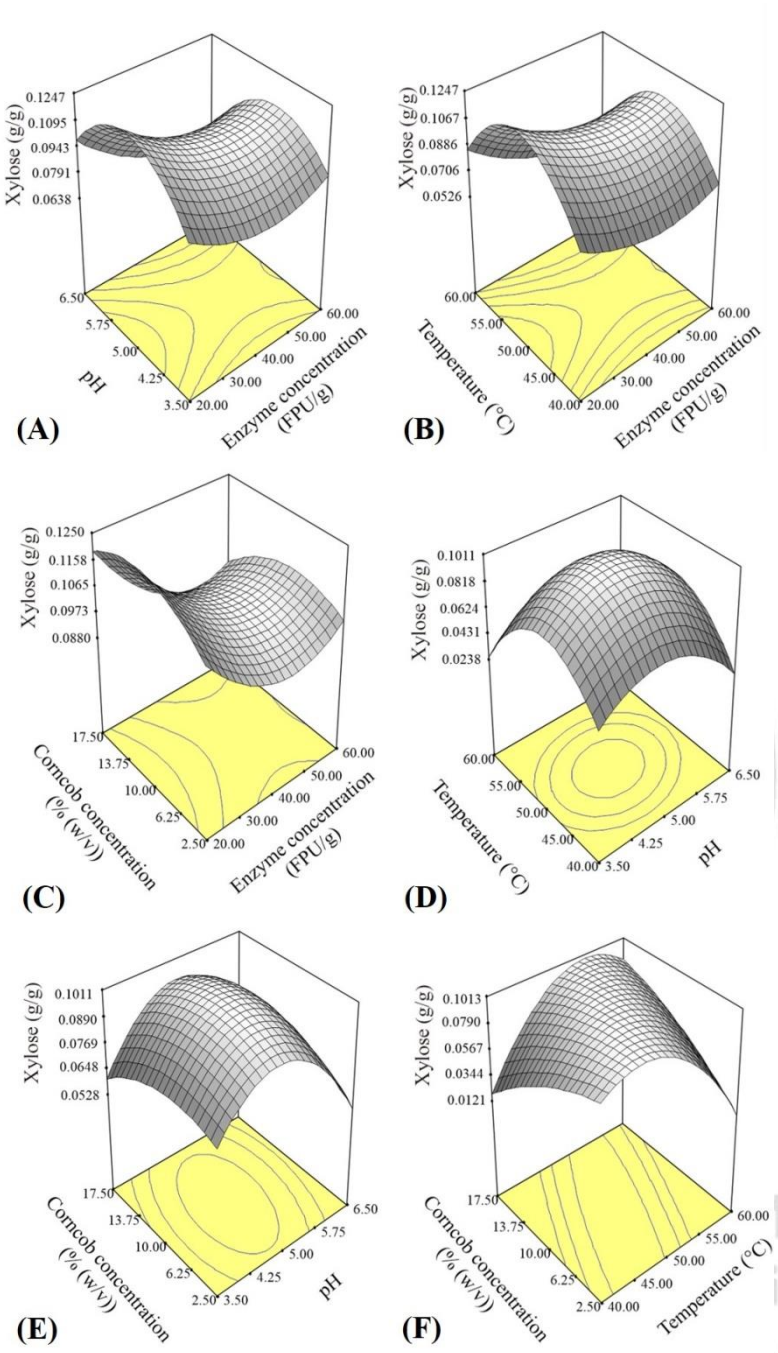

Figure 5.5 Xylose production from cellulose-rich corncob in three-dimensional graphic for quadratic response surface optimization. The comparison was made between (A) enzyme concentration and pH value (X_1X_2), (B) enzyme concentration and temperature (X_1X_3), (C) enzyme concentration and cellulose-rich corncob concentration (X_1X_4), (D) pH value and temperature (X_2X_3), (E) pH and cellulose-rich corncob concentration (X_2X_4) and (F) temperature and cellulose-rich corncob concentration (X_3X_4).

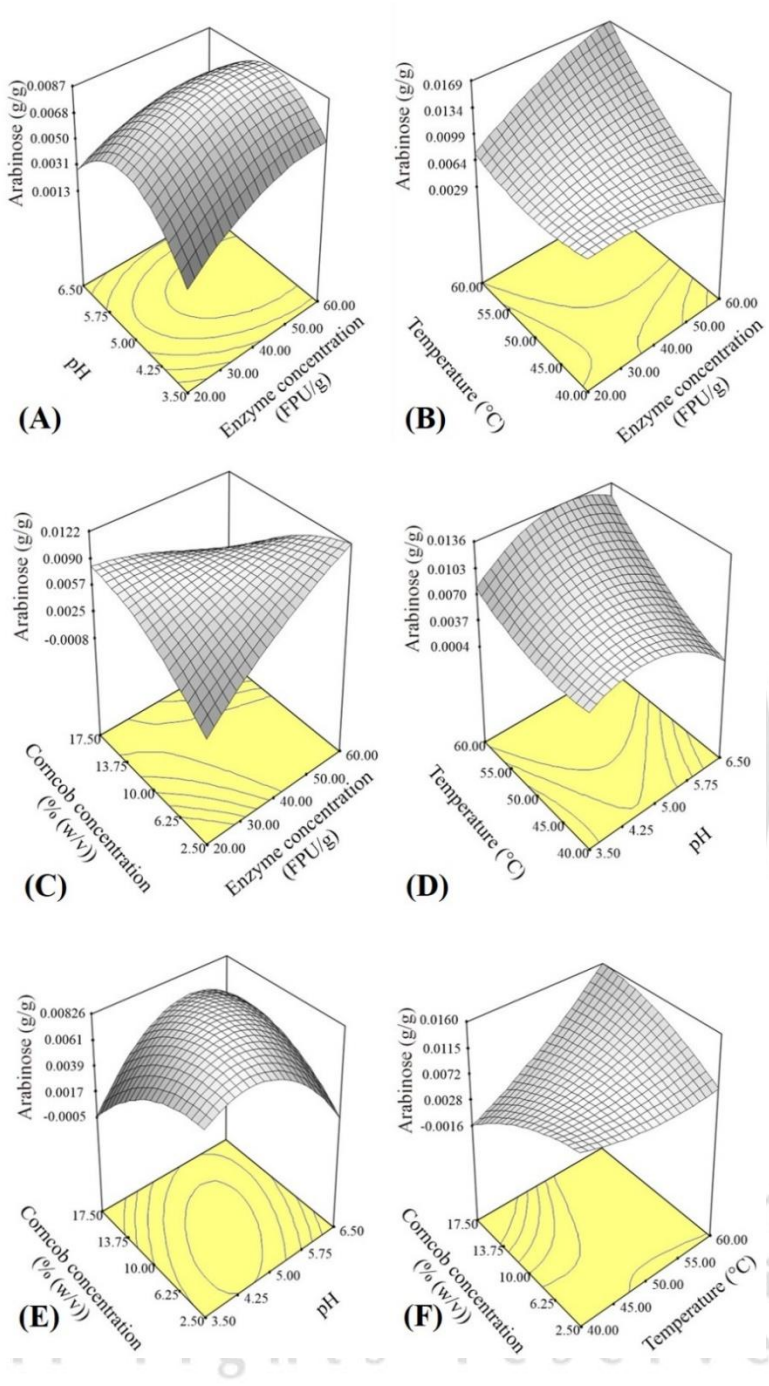

Figure 5.6 Arabinose production from cellulose-rich corncob in three-dimensional graphic for quadratic response surface optimization. The comparison was made between (A) enzyme concentration and pH value (X_1X_2), (B) enzyme concentration and temperature (X_1X_3), (C) enzyme concentration and cellulose-rich corncob concentration (X_1X_4), (D) pH value and temperature (X_2X_3), (E) pH and cellulose-rich corncob concentration (X_2X_4) and (F) temperature and cellulose-rich corncob concentration (X_3X_4).

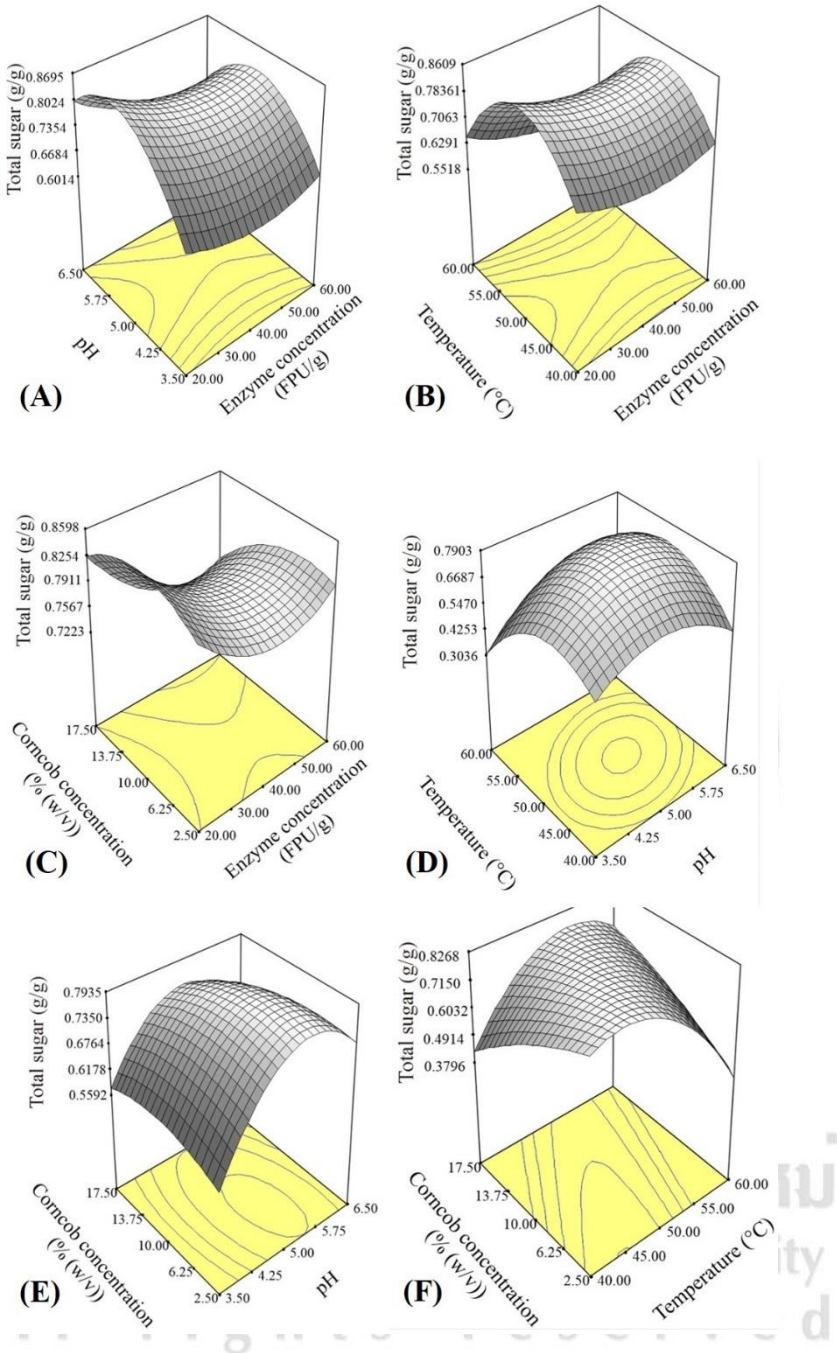

Figure 5.7 Total sugar production from cellulose-rich corncob in three-dimensional graphic for quadratic response surface optimization. The comparison was made between (A) enzyme concentration and pH value (X_1X_2), (B) enzyme concentration and temperature (X_1X_3), (C) enzyme concentration and cellulose-rich corncob concentration (X_1X_4), (D) pH value and temperature (X_2X_3), (E) pH and cellulose-rich corncob concentration (X_2X_4) and (F) temperature and cellulose-rich corncob concentration (X_3X_4).

Table 5.3 Experimental design and response for optimization of glucose, xylose, arabinose and total sugar production from cellulose-rich corncob.

Std. order	Factors				Glucose (g/g)		Xylose (g/g)		Arabinose (g/g)		Total sugar (g/g)	
	X_1 *	X_2 **	X_3 ***	X_4 ****	Actual value	Predicted value	Actual value	Predicted value	Actual value	Predicted value	Actual value	Predicted value
1	20	3.5	50	10	0.56	0.56	0.08	0.09	0.00	0.00	0.64	0.65
2	60	3.5	50	10	0.59	0.55	0.08	0.09	0.01	0.01	0.68	0.64
3	20	6.5	50	10	0.68	0.70	0.10	0.10	0.00	0.00	0.79	0.80
4	60	6.5	50	10	0.64	0.62	0.08	0.07	0.00	0.00	0.72	0.69
5	40	5	40	2.5	0.72	0.69	0.07	0.09	0.01	0.01	0.80	0.79
6	40	5	60	2.5	0.38	0.36	0.01	0.01	0.01	0.01	0.40	0.38
7	40	5	40	17.5	0.41	0.41	0.02	0.02	0.00	0.00	0.43	0.43
8	40	5	60	17.5	0.57	0.58	0.10	0.08	0.02	0.02	0.69	0.67
9	20	5	50	2.5	0.71	0.72	0.10	0.11	0.00	0.00	0.81	0.83
10	60	5	50	2.5	0.62	0.69	0.12	0.10	0.01	0.01	0.75	0.80

Table 5.3 Experimental design and response for optimization of glucose, xylose, arabinose and total sugar production from cellulose-rich corncob. (continued)

Std. order	Factors				Glucose (g/g)		Xylose (g/g)		Arabinose (g/g)		Total sugar (g/g)	
	X_1*	X_2**	X_3***	X_4****	Actual value	Predicted value	Actual value	Predicted value	Actual value	Predicted value	Actual value	Predicted value
11	20	5	50	17.5	0.71	0.70	0.09	0.12	0.01	0.01	0.80	0.82
12	60	5	50	17.5	0.59	0.64	0.09	0.09	0.00	0.00	0.68	0.73
13	40	3.5	40	10	0.46	0.48	0.06	0.04	0.00	0.00	0.52	0.53
14	40	6.5	40	10	0.50	0.46	0.03	0.02	0.00	0.00	0.53	0.48
15	40	3.5	60	10	0.17	0.27	0.01	0.03	0.01	0.01	0.19	0.30
16	40	6.5	60	10	0.47	0.51	0.00	0.03	0.01	0.01	0.48	0.55
17	20	5	40	10	0.58	0.62	0.10	0.08	0.01	0.01	0.69	0.71
18	60	5	40	10	0.59	0.60	0.05	0.07	0.00	0.00	0.64	0.67
19	20	5	60	10	0.60	0.55	0.12	0.09	0.01	0.01	0.73	0.65
20	60	5	60	10	0.57	0.49	0.05	0.06	0.02	0.02	0.64	0.57

Table 5.3 Experimental design and response for optimization of glucose, xylose, arabinose and total sugar production from cellulose-rich corncob. (continued)

Std. order	Factors				Glucose (g/g)		Xylose (g/g)		Arabinose (g/g)		Total sugar (g/g)	
	X_1*	X_2**	X_3***	X_4****	Actual value	Predicted value	Actual value	Predicted value	Actual value	Predicted value	Actual value	Predicted value
21	40	3.5	50	2.5	0.52	0.48	0.08	0.07	0.01	0.01	0.61	0.56
22	40	6.5	50	2.5	0.65	0.66	0.06	0.05	0.00	0.00	0.71	0.71
23	40	3.5	50	17.5	0.56	0.52	0.07	0.06	0.00	0.00	0.63	0.58
24	40	6.5	50	17.5	0.55	0.55	0.07	0.07	0.01	0.01	0.63	0.62
25	40	5	50	10	0.71	0.68	0.11	0.10	0.01	0.01	0.83	0.79
26	40	5	50	10	0.70	0.68	0.10	0.10	0.01	0.01	0.81	0.79
27	40	5	50	10	0.65	0.68	0.10	0.10	0.01	0.01	0.76	0.79
28	40	5	50	10	0.66	0.68	0.08	0.10	0.00	0.01	0.74	0.79
29	40	5	50	10	0.66	0.68	0.12	0.10	0.01	0.01	0.78	0.79

*X_1: enzyme concentration (FPU/g_{CRC}); **X_2: pH; ***X_3: temperature (°C); ****X_4: CRC concentration (% (w/v))

Table 5.4 Analysis of variance (ANOVA) for response surface quadratic model of glucose, xylose, arabinose and total sugar production.

Coefficient	**Glucose ($g_{glucose}/g_{CRC}$)**		**Xylose (g_{xylose}/g_{CRC})**		**Arabinose ($g_{arabinose}/g_{CRC}$)**		**Total sugar (g_{sugar}/g_{CRC})**	
	p-value	*F*-value	*p*-value	*F*-value	*p*-value	*F*-value	*p*-value	*F*-value
Model	0.0001[a]	8.81	0.0121[a]	3.54	<0.0001[a]	11.17	<0.0001[a]	10.70
X_1	0.2194	1.65	0.1546	2.26	0.0203[a]	6.85	0.1279	2.62
X_2	0.0045[a]	11.39	0.5752	0.33	1.0000	0.00	0.0146[a]	7.75
X_3	0.0180[a]	7.18	0.6532	0.21	<0.0001[a]	42.79	0.0348[a]	5.46
X_4	0.2795	1.27	0.9900	1.6×10^{-4}	0.3262	1.04	0.3170	1.08
X_1^2	0.1339	2.53	0.1420	2.42	0.1385	2.47	0.0724	3.78
X_2^2	0.0003[a]	23.51	0.0056[a]	10.67	0.0005[a]	19.86	<0.0001[a]	32.18
X_3^2	<0.0001[a]	46.22	0.0004[a]	21.71	0.0942	3.22	<0.0001[a]	59.60
X_4^2	0.3038	1.14	0.3012	1.15	0.0325[a]	5.63	0.1756	2.04
X_1X_2	0.5265	0.42	0.6194	0.26	0.4626	0.57	0.4419	0.63

Table 5.4 Analysis of variance (ANOVA) for response surface quadratic model of glucose, xylose, arabinose and total sugar production. (continued)

Coefficient	**Glucose ($g_{glucose}/g_{CRC}$)**		**Xylose (g_{xylose}/g_{CRC})**		**Arabinose ($g_{arabinose}/g_{CRC}$)**		**Total sugar (g_{sugar}/g_{CRC})**	
	p-value	*F*-value	*p*-value	*F*-value	*p*-value	*F*-value	*p*-value	*F*-value
X_1X_3	0.7161	0.14	0.6654	0.20	0.0056 [a]	10.71	0.7042	0.15
X_1X_4	0.7848	0.078	0.7453	0.11	0.0002 [a]	25.35	0.6005	0.29
X_2X_3	0.0301 [a]	5.82	0.6044	0.28	0.0636	4.06	0.0304 [a]	5.79
X_2X_4	0.2149	1.69	0.6044	0.28	0.0151 [a]	7.67	0.4013	0.75
X_3X_4	0.0004 [a]	21.53	0.0080 [a]	9.56	0.0002 [a]	25.35	<0.0001 [a]	29.60
Lack of fit	0.0637	5.17	0.115	3.59	0.3200	0.94	3.9200	0.10
C.V.	9.31		30.75		32.72		9.21	
R^2 of model	0.8980		0.7798		0.9178		0.9145	

[a] Significant at $p \leq 0.05$

5.3.4 Separate hydrolysis and fermentation (SHF) process of bioethanol production

To determine the effect of temperature on bioethanol production by *S. cerevisiae* and *C. glabrata* KY618709, the SHF process was conducted under limited oxygen conditions at a temperature range of 35–42°C (Figure 5.8–5.11) the ethanol yields from *S. cerevisiae* and *C. glabrata* KY618709 at different temperatures are shown in Table 5.5. The results reveal that ethanol production via SHF using *S. cerevisiae* strongly depends on temperature. The ethanol concentration decreased from 20.45 to 9.64 g/L when the temperature was elevated to 42°C, while bioethanol concentration of 20.07–21.92 g/L from strain KY618709 at various temperatures were not significantly different. Moreover, the production yield and theoretical yield from *S. cerevisiae* decreased at increased temperatures (Table 5.5). In agreement with previous reports, the optimum temperature for ethanol production by *S. cerevisiae* was between 28 and 30°C (Mohd Azhar et al., 2017; Zabed et al., 2017). Surprisingly, changing the temperature within the range 35–42°C did not show any effects on ethanol yield from strain KY618709. Recent studies have shown that *C. glabrata* can survive and produce ethanol at temperatures between 30 and 42°C (Watanabe et al., 2009; Watanabe et al., 2010).

The time course of bioethanol production via SHF of *S. cerevisiae* and strain KY618709 at 40 and 42°C is shown in Figure 5.10 and 5.11. For the SHF process at 40°C, glucose was rapidly and completely consumed by strain KY618709 after 48 h, while *S. cerevisiae* could not completely utilize glucose within a 120 h fermentation period (Figure 5.10). Moreover, the ethanol production rate of *S. cerevisiae* was relatively low. At 40°C, 22.98 g/L of ethanol was obtained from strain KY618709, which was dramatically higher than the amount of ethanol produced by *S. cerevisiae* (14.16 g/L). When SHF of the two yeast strains was carried out at 42°C, the glucose consumption rate was increased within 72 h of fermentation and then the consumption rate was steady (Figure 5.11). Strain KY618709 consumed more than 90% of the glucose and had an ethanol concentration of 20.64 g/L. However, Dyartanti et al. (2015) reported that the SHF process requires a lot of equipment and is costly. Hence, SSF has been developed to reduce operation costs and time, reduce inhibition of end-products and increase bioethanol productivity (Cha et al., 2015; Dyartanti et al., 2015; Watanabe et al., 2012). However, a difference between the optimum temperature for enzymatic hydrolysis and that for growth of ethanolic microorganisms is the most

common limitation of SSF (Narra et al., 2015). Therefore, the effect of temperature on bioethanol production by thermotolerant strain KY618709 was investigated in order to get rid of the limitation problem in SSF.

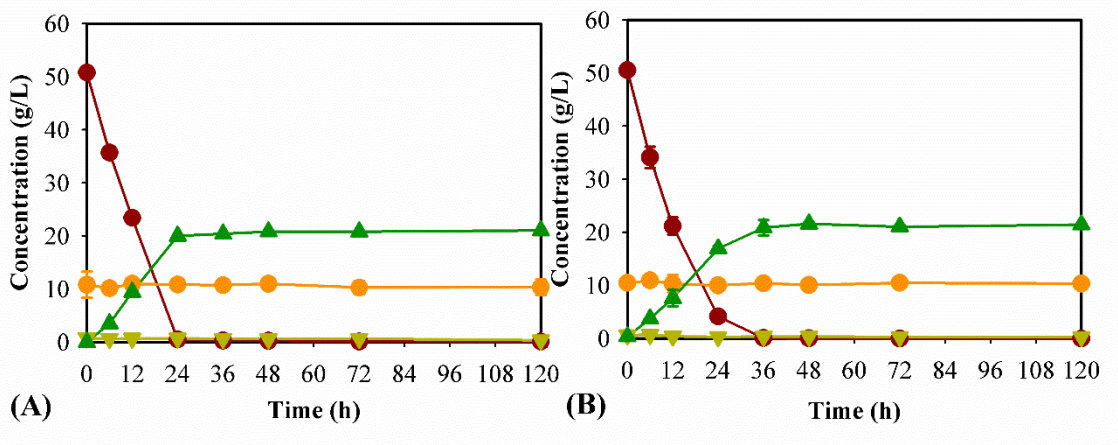

Figure 5.8 Time course of bioethanol production from cellulose-rich corncob hydrolysate by (A) *Sacchromyces cerevisiae* and by (B) *Candida glabrata* KY618709 at 35°C in 100-mL bottles via separate hydrolysis and fermentation (SHF). (●: glucose; ●: xylose; ▼: arabinose; ▲: ethanol)

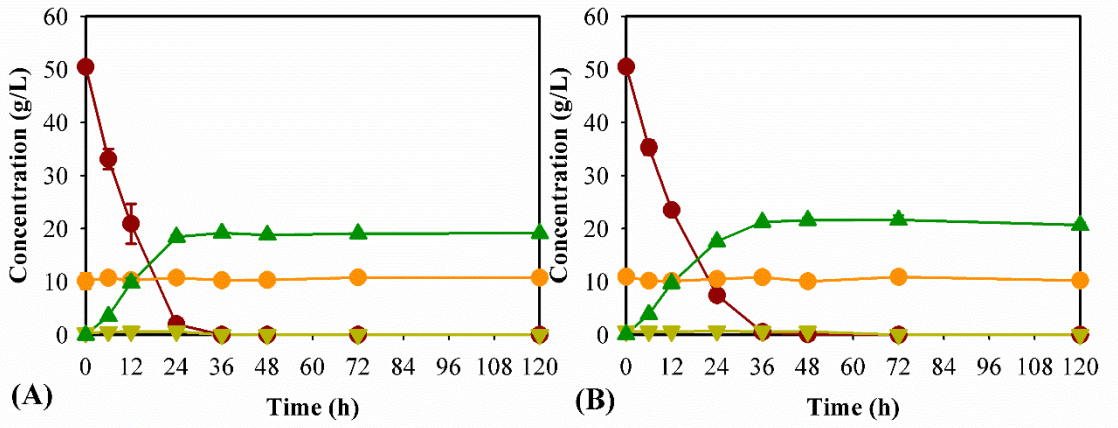

Figure 5.9 Time course of bioethanol production from cellulose-rich corncob hydrolysate by (A) *Saccharomyces cerevisiae* and by (B) *Candida glabrata* KY618709 at 37°C in 100-mL bottles via separate hydrolysis and fermentation (SHF). (●: glucose; ●: xylose; ▼: arabinose; ▲: ethanol)

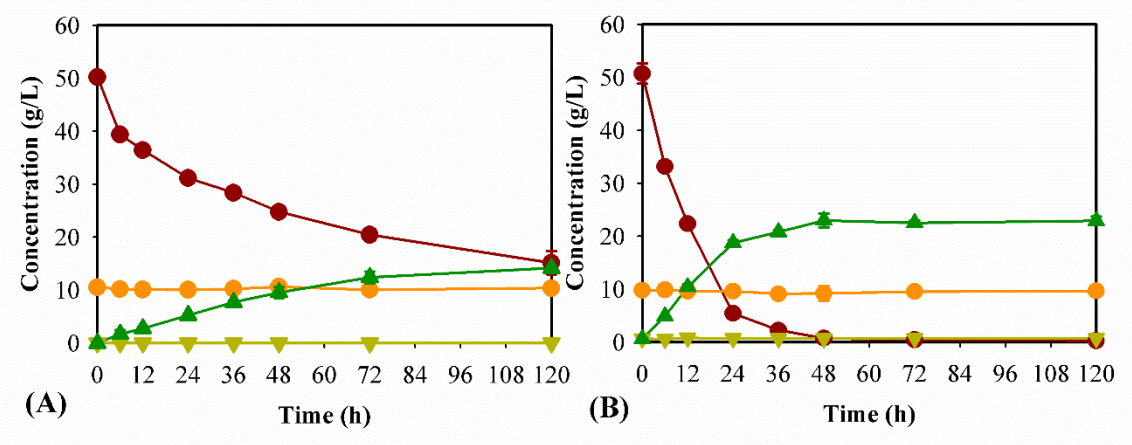

Figure 5.10 Time course of bioethanol production from cellulose-rich corncob hydrolysate by (A) *Saccharomyces cerevisiae* and by (B) *Candida glabrata* KY618709 at 40°C in 100-mL bottles via separate hydrolysis and fermentation (SHF). (●: glucose; ●: xylose; ▼: arabinose; ▲: ethanol)

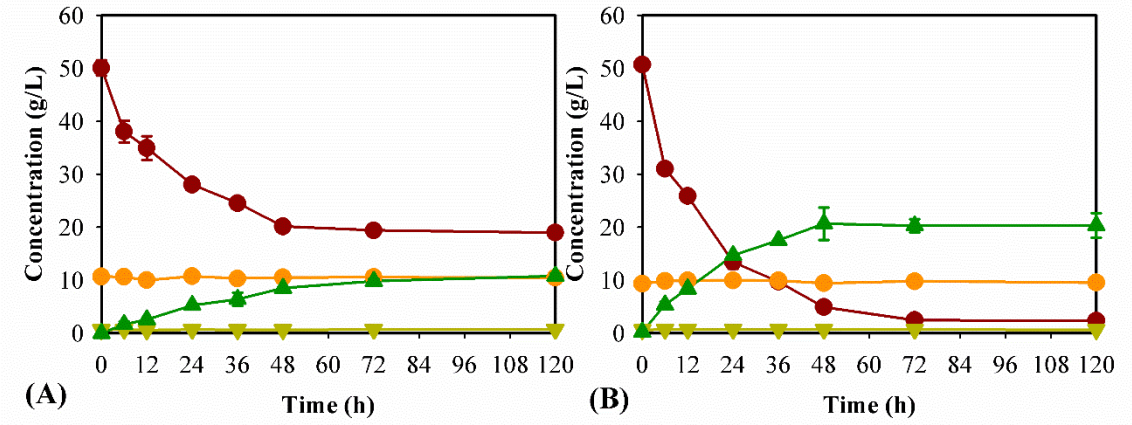

Figure 5.11 Time course of bioethanol production from cellulose-rich corncob hydrolysate by (A) *Saccharomyces cerevisiae* and by (B) *Candida glabrata* KY618709 at 42°C in 100-mL bottles via separate hydrolysis and fermentation (SHF). (●: glucose; ●: xylose; ▼: arabinose; ▲: ethanol)

5.3.5 Simultaneous saccharification and fermentation (SSF) process of bioethanol production

5.3.5.1 Laboratory scale in 100-mL bottles

SSF processes using thermotolerant yeast and commercial yeast were compared, to find an alternative process to reduce overall process time and equipment. Temperature is considered one of the most crucial factors for SSF processes using LCMs. Generally, the optimal temperature for enzymatic hydrolysis is around 50°C, while the optimal temperature for ethanol fermentation by *S. cerevisiae* is around 30°C (Huang et al., 2017). The effect of temperature on ethanol production by both strains of yeast was examined. As shown in Figure 5.12, when using *S. cerevisiae*, the ethanol titers were obviously lower than those for strain KY618709. Ethanol yields of *S. cerevisiae* depleted gradually with a rise in temperature (Figure 5.15). On the other hand, SSF using strain KY618709 at 35–42°C was not significantly different, with an ethanol concentration of 21.05–22.35 g/L (Table 5.5). The changes in glucose, xylose, arabinose and ethanol during SSF using *S. cerevisiae* and strain KY618709 at 40 and 42°C are shown in Figure 5.14 and 5.15.

At 40°C, glucose was immediately fermented by strain KY618709 and ethanol was rapidly generated. In contrast, glucose remained and could not be completely utilized by *S. cerevisiae* (Figure 5.14). Moreover, at 42°C, released glucose can be immediately fermented by strain KY618709, while, *S. cerevisiae* showed a slow consumption rate of glucose in order to continuously accumulate it in the fermentation medium (Figure 5.15). Hence, the ethanol concentration from *S. cerevisiae* was relatively low at only 7.44 g/L. The results revealed that temperature has a negative effect on sugar consumption and ethanol fermentation by *S. cerevisiae*. Generally, *S. cerevisiae* is a mesophilic microorganism. High temperature can affect cell morphology and physiology, as well as ethanol fermentation efficiency (Choudhary et al., 2017). However, the isolated yeast strain KY618709 showed a satisfactory performance in ethanol fermentation at elevated temperatures (40–42°C). This is in agreement with the report of Watanabe et al. (2009), who revealed that *C. glabrata* is a promising thermotolerant ethanol-producing yeast strain for bioethanol production. They suggested that *C. glabrata* is different from several *Candida* strains because it is able to grow and produce ethanol under oxygen-limited conditions. For ethanol production, *C. glabrata* has similar characteristics to

those of *S. cerevisiae* (Watanabe et al., 2009; Watanabe et al., 2010). It has a greater ability to tolerate both elevated temperature and low pH values than *S. cerevisiae* (Choudhary et al., 2016).

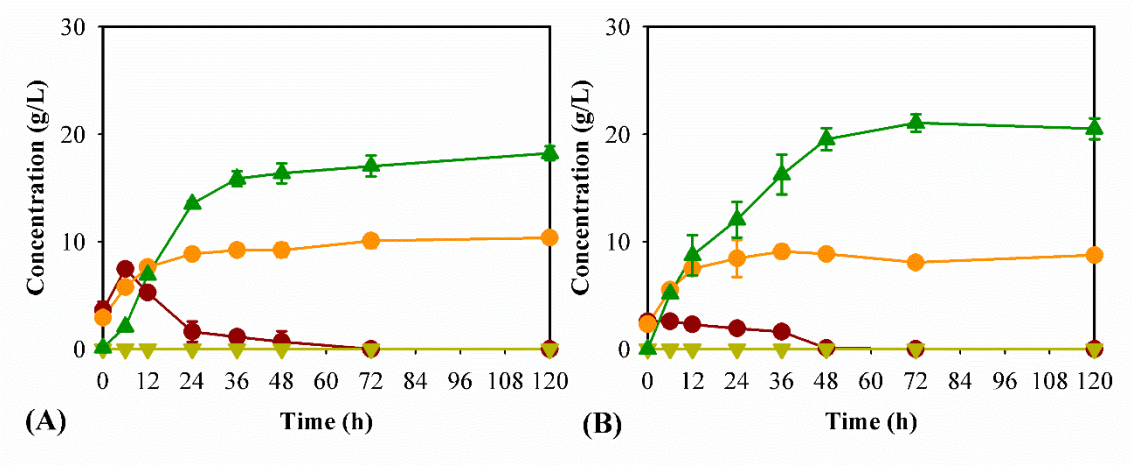

Figure 5.12 Time course of bioethanol production from cellulose-rich corncob by (A) *Saccharomyces cerevisiae* and by (B) *Candida glabrata* KY618709 at 35°C in 100-mL bottles via simultaneous saccharification and fermentation (SSF). (●: glucose; ●: xylose; ▼: arabinose; ▲: ethanol)

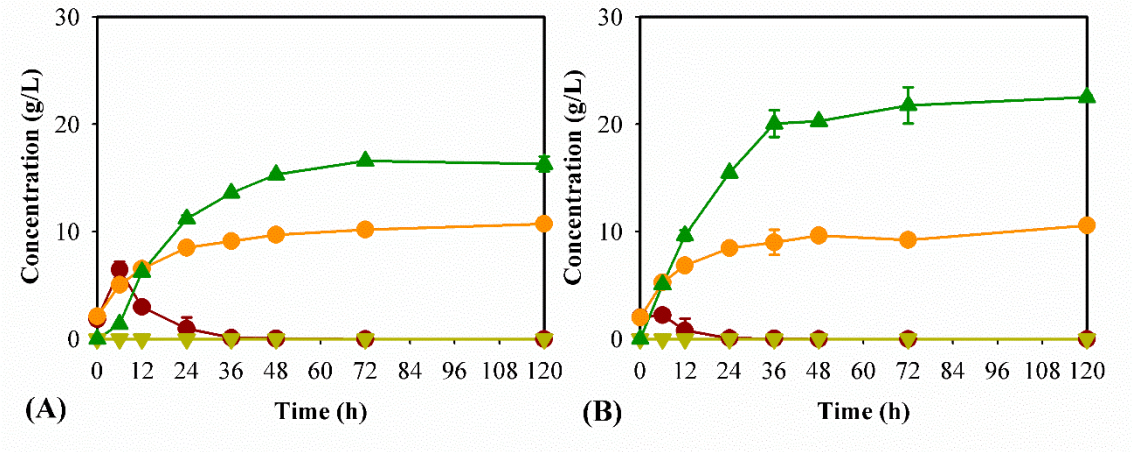

Figure 5.13 Time course of bioethanol production from cellulose-rich corncob by (A) *Saccharomyces cerevisiae* and by (B) *Candida glabrata* KY618709 at 37°C in 100-mL bottles via simultaneous saccharification and fermentation (SSF). (●: glucose; ●: xylose; ▼: arabinose; ▲: ethanol)

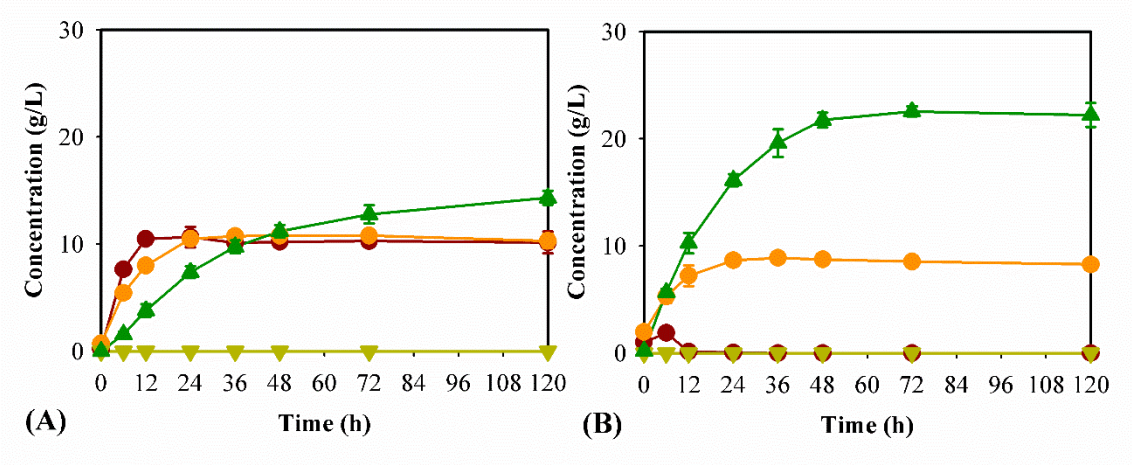

Figure 5.14 Time course of bioethanol production from cellulose-rich corncob by (A) *Saccharomyces cerevisiae* and by (B) *Candida glabrata* KY618709 at 40°C in 100-mL bottles via simultaneous saccharification and fermentation (SSF). (●: glucose; ●: xylose; ▼: arabinose; ▲: ethanol)

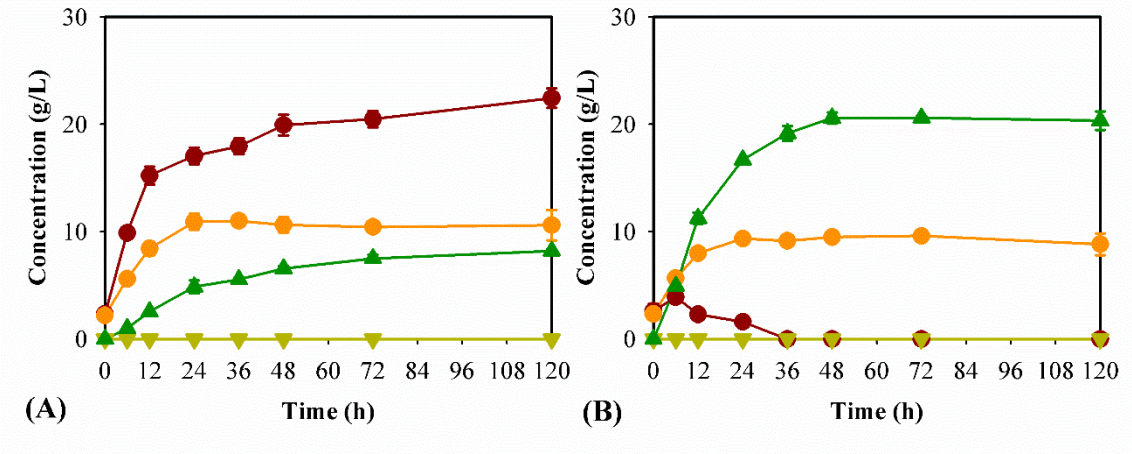

Figure 5.15 Time course of bioethanol production from cellulose-rich corncob by (A) *Saccharomyces cerevisiae* and by (B) *Candida glabrata* KY618709 at 42°C in 100-mL bottles via simultaneous saccharification and fermentation (SSF). (●: glucose; ●: xylose; ▼: arabinose; ▲: ethanol)

At laboratory scale, strain KY618709 showed a higher ethanol titer, ethanol productivity and ethanol yield than *S. cerevisiae* in both processes (Table 5.5). Although ethanol production by strain KY618709 in SHF and SSF was not significantly different, the overall process time for ethanol fermentation via SSF was reduced from 168 h in SHF to 72 h.

5.3.5.2 Ethanol production in a 5-L bioreactor

The ethanol production via SSF with 7.8% CRC loading at 40°C was performed in a 5-L bioreactor. To increase the final ethanol concentration, fed-batch SSF with 11.7% and 15.6% CRC was also examined. Figure 5.16 shows the time course of bioethanol production via SSF and fed-batch SSF by the thermotolerant yeast strain *C. glabrata* KY618709. The results showed that strain KY618709 can utilize almost all glucose to produce ethanol, not only at 7.8% CRC loading but also at 11.7% CRC loading. SSF with 7.8% CRC loading produced ethanol up to 21.48 g/L with a yield of 0.269 g/g, productivity of 0.298 g/L/h and 91% theoretical yield (Figure 5.16 and Table 5.5). While, Kossatz et al. (2017) reported the ethanol production from triticale straw conducted in a bioreactor via SSF process with 15% solid, 37°C, 144 h using commercial *S. cerevisiae* and cellulase. Even the ethanol concentration of 29 g/L was higher than strain KY618709, a relatively low ethanol yield of 0.193 g/g with a productivity of 0.203 g/L/h and theoretical yield of 85% were obtained.

The ethanol production from strain KY618709 via fed-batch SSF with 11.7 and 15.6% CRC loading was also investigated. The highest ethanol concentration of 37.0 g/L was obtained from 15.6% CRC loading while the maximal ethanol yield 0.624 g/g, productivity of 0.326 g/L/h with the theoretical yield 89% were obtained from 11.7% CRC loading which were higher than those obtained from other processes (Table 5.5). The ethanol production from various type of feedstock via fed-batch SSF in bioreactor has been investigated. For example, Hoyer et al. (2010) reported the bioethanol production from spruce wood using *S. cerevisiae* in 2-L bioreactor with 14% solid and 37°C. The ethanol concentration of 25 g/L and theoretical yield of 70% were obtained. Meanwhile, Pessani et al. (2011) reported ethanol production from switchgrass by a thermotolerant yeast *Kluyveromyces marxianus* IMB3. The low ethanol concentration of 27 g/L, corresponding to the theoretical yield 80% were achieved at 12% solid and 45°C.

Even though the ethanol titer of fed-batch SSF with 12–15% solid from our study and other mentioned reports were relative high at 29–40 g/L, the ethanol productivity and theoretical yields were lower than those for 11.7% of CRC loading. The obtained results of this study are in accordance with previously reports. These also indicate that

the solid loading is considering as a limiting factor for both of enzymatic hydrolysis and ethanol fermentation. Previous studies found that a high solid loading can increase inhibitor formation, osmotic pressure and stress from low water activity during ethanol fermentation by yeast (Jørgensen et al., 2007; Kossatz et al., 2017). Generally, the optimal solid concentration for effective cellulose conversion is 10% (Jørgensen et al., 2007)

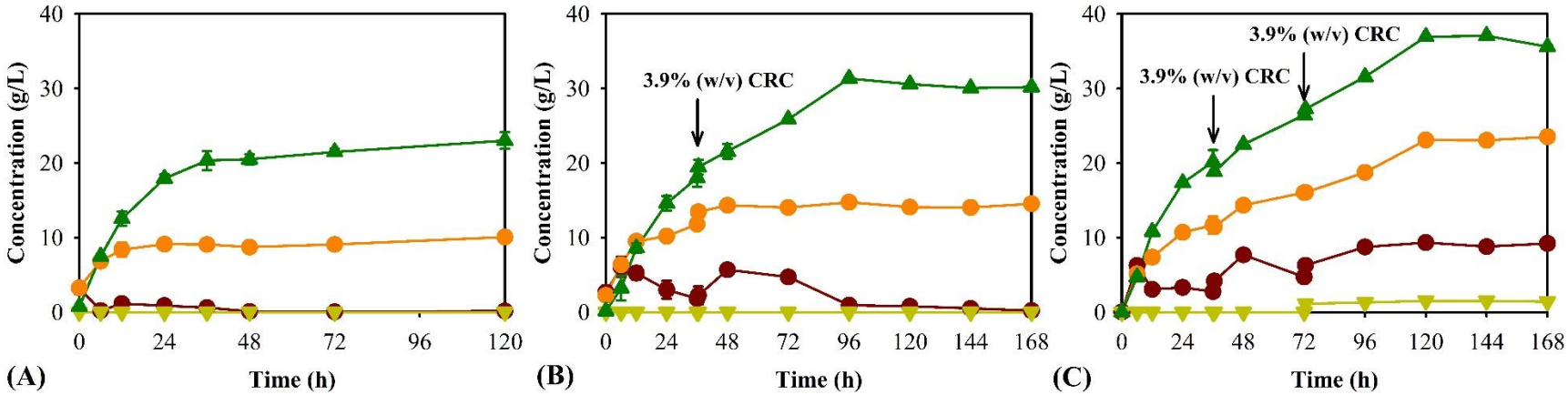

Figure 5.16 Time course of bioethanol production via simultaneous saccharification and fermentation with (A) 7.8% (w/v) cellulose-rich corncob, (B) fed-batch simultaneous saccharification and fermentation with 11.7% (w/v) cellulose-rich corncob and (C) fed-batch simultaneous saccharification and fermentation with 15.6% (w/v) cellulose-rich corncob by the thermotolerant yeast *Candida glabrata* KY618709 in a 5-L bioreactor at 40°C. (●: glucose; ●: xylose; ▼: arabinose; ▲: ethanol)

Table 5.5 Ethanol production via separate hydrolysis and fermentation (SHF), simultaneous saccharification and fermentation (SSF) and fed-batch simultaneous saccharification and fermentation (fed-batch SSF) by *Candida glabrata* KY618709 compare with commercial yeast *Saccharomyces cerevisiae*.

Process	Yeast strain	Temperature (°C)	CRC loading (% (w/v))	Time (h)	C_{EtOH}* (g/L)	Q_p** (g/L/h)	Y_{EtOH}*** ($g_{EtOH}/g_{cellulose}$)	Conversion of cellulose to ethanol (%)	Y**** (%)
Laboratory scale									
SHF	KY618709	35	7.8	72	20.67 ± 0.37^{cd}	0.287 ± 0.004^{bc}	0.357 ± 0.005^{ab}	62.80 ± 0.88^{abc}	87.76 ± 1.22^{abc}
		37	7.8	72	21.54 ± 0.85^{c}	0.299 ± 0.008^{ab}	0.372 ± 0.011^{a}	65.45 ± 1.85^{a}	91.46 ± 2.59^{a}
		40	7.8	72	21.92 ± 0.40^{c}	0.304 ± 0.004^{ab}	0.378 ± 0.004^{a}	66.60 ± 0.79^{a}	93.07 ± 1.11^{a}
		42	7.8	72	20.07 ± 1.24^{cd}	0.279 ± 0.012^{bc}	0.346 ± 0.015^{ab}	60.98 ± 2.69^{abc}	85.22 ± 3.76^{abc}
	S. cerevisiae	35	7.8	72	20.45 ± 0.09^{cd}	0.284 ± 0.000^{bc}	0.353 ± 0.000^{ab}	62.14 ± 0.02^{abc}	86.84 ± 0.03^{abc}
		37	7.8	72	18.85 ± 0.11^{de}	0.262 ± 0.003^{cd}	0.325 ± 0.003^{bc}	57.25 ± 0.56^{bcd}	80.01 ± 0.79^{bcd}
		40	7.8	72	12.30 ± 1.09^{g}	0.171 ± 0.010^{f}	0.212 ± 0.013^{d}	37.34 ± 2.29^{e}	52.18 ± 3.20^{e}
		42	7.8	72	9.64 ± 0.45^{h}	0.134 ± 0.004^{g}	0.166 ± 0.005^{e}	29.26 ± 0.85^{f}	40.89 ± 1.19^{f}
SSF	KY618709	35	7.8	72	21.05 ± 0.81^{cd}	0.292 ± 0.008^{bc}	0.363 ± 0.010^{ab}	63.93 ± 1.75^{ab}	89.35 ± 2.45^{ab}
		37	7.8	72	21.76 ± 1.70^{c}	0.302 ± 0.017^{ab}	0.375 ± 0.021^{a}	66.09 ± 3.64^{a}	92.36 ± 5.09^{a}
		40	7.8	72	22.35 ± 0.46^{c}	0.310 ± 0.002^{ab}	0.385 ± 0.002^{a}	67.88 ± 0.40^{a}	94.86 ± 0.56^{a}
		42	7.8	72	20.60 ± 0.17^{cd}	0.286 ± 0.002^{bc}	0.355 ± 0.003^{ab}	62.57 ± 0.51^{abc}	87.44 ± 0.71^{abc}

Table 5.5 Ethanol production via separate hydrolysis and fermentation (SHF), simultaneous saccharification and fermentation (SSF) and fed-batch simultaneous saccharification and fermentation (fed-batch SSF) by *Candida glabrata* KY618709 compare with commercial yeast *Saccharomyces cerevisiae*. (continued)

Process	**Yeast strain**	**Temperature (°C)**	**CRC loading (% (w/v))**	**Time (h)**	**C_{EtOH} * (g/L)**	**Q_p ** (g/L/h)**	**Y_{EtOH} *** ($g_{EtOH}/g_{cellulose}$)**	**Conversion of cellulose to ethanol (%)**	**Y**** (%)**
SSF	*S. cerevisiae*	35	7.8	72	16.91 ± 1.30^{ef}	0.235 ± 0.014^{de}	0.292 ± 0.018^{c}	51.36 ± 3.17^{d}	71.77 ± 4.42^{d}
		37	7.8	72	16.57 ± 0.04^{f}	0.230 ± 0.000^{e}	0.286 ± 0.000^{c}	50.34 ± 0.08^{d}	70.36 ± 0.11^{d}
		40	7.8	72	12.69 ± 3.18^{g}	0.176 ± 0.030^{f}	0.219 ± 0.038^{d}	38.55 ± 6.63^{e}	53.88 ± 9.26^{e}
		42	7.8	72	7.44 ± 0.33^{i}	0.103 ± 0.004^{g}	0.128 ± 0.005^{f}	22.62 ± 0.87^{g}	31.61 ± 1.22^{g}
Bioreactor scale									
SSF	KY618709	40	7.8	72	21.48 ± 0.02^{c}	0.298 ± 0.006^{ab}	0.371 ± 0.000^{a}	65.26 ± 0.05^{a}	91.21 ± 0.06^{a}
Fed-batch	KY618709	40	11.7	96	31.32 ± 0.24^{b}	0.326 ± 0.003^{a}	0.360 ± 0.003^{ab}	63.43 ± 0.48^{abc}	88.63 ± 0.67^{abc}
SSF		40	15.6	120	36.99 ± 0.06^{a}	0.308 ± 0.001^{ab}	0.319 ± 0.001^{bc}	56.18 ± 0.10^{cd}	78.51 ± 0.14^{cd}

*C_{EtOH}: maximum ethanol concentration; **Q_p: ethanol production rate; ***Y_{EtOH}: ethanol yield; ****Y: theoretical ethanol yield.

Values are presented as mean ± standard deviation, $n = 3$. Data with the same superscript in the same column are not significantly different at $p \leq 0.05$ (different kinetic values were compared between fermentation processes with each yeast strain). The level of significance was tested by Duncan's multiple range testat $p \leq 0.05$.

5.3.6 Mass balance of the integrated process for xylooligosaccharides and bioethanol production

Mass balances for the integrated process for XO and bioethanol production (based on 1 kg of raw corncob) are shown in Figure 5.17. After the XO production process, 115 g of hydrolysis products from the in-house thermostable endo-xylanase (liquid phase) and 515 g of CRC (solid phase) was obtained from 1.0 kg of raw corncob. The liquid phase of hydrolysis products from this in-house xylanase included 79 g of XOs, 21 g of xylose and 15 g of arabinose. The 515 g of solid phase contained 383 g of cellulose, 68 g of hemicellulose and 64 g of other components.

Not only the yields of XOs and bioethanol, but the content of cellulose, hemicellulose and lignin of raw corncob, KOH-treated corncob and CRC were also concerned. Raw corncob contained 42.62% cellulose, 38.82% hemicellulose, 8.69% lignin and 9.88% other components. After KOH pretreatment, the cellulose contents in treated-corncob were moderately increased to 64.46%, while hemicellulose was slightly decreased to 31.27%. However, the lignin content was dramatically decreased to only 2.52% indicating that the ester bonds of hemicellulose-lignin complex are degraded in order to solubilize of lignin in alkali solution (Chen et al., 2017). After XO production step, CRC remained only 13.20% hemicellulose. The decreasing in hemicellulose was agreeable to the XO yield of 18.07%. Furthermore, the CRC reached in high cellulose content of 74.33% indicating to be a promising substrate for bioethanol production. However, further study is required to completely valorize of lignin, one of major components found in LCMs. Hence, the liquid fraction containing high lignin content from KOH pretreatment step might be a potential substrate for high value products such as furfural, lignosulfonate, vanillin and syringaldehyde (Tan et al., 2016; Zhu et al, 2013).

Using the SHF process, 410 g of fermentable sugar, 338 g of glucose (82.44%), 66 g of xylose (16.10%) and 6 g of arabinose (1.46%) was obtained. At laboratory-scale SHF with strain KY618709, the highest ethanol yield of 145 g, 0.281 $g_{ethanol}/g_{CRC}$ or 0.378 $g_{ethanol}/g_{cellulose}$ was obtained at 40°C. Laboratory-scale SSF with strain KY618709 at 40°C resulted in the highest ethanol yield of 148 g, 0.287 $g_{ethanol}/g_{CRC}$ or 0.385

$g_{ethanol}/g_{cellulose}$, while for scaled-up fed-batch SSF with 11.7% CRC loading, 138 g of ethanol, 0.268 $g_{ethanol}/g_{CRC}$ or 0.360 $g_{ethanol}/g_{cellulose}$ was obtained.

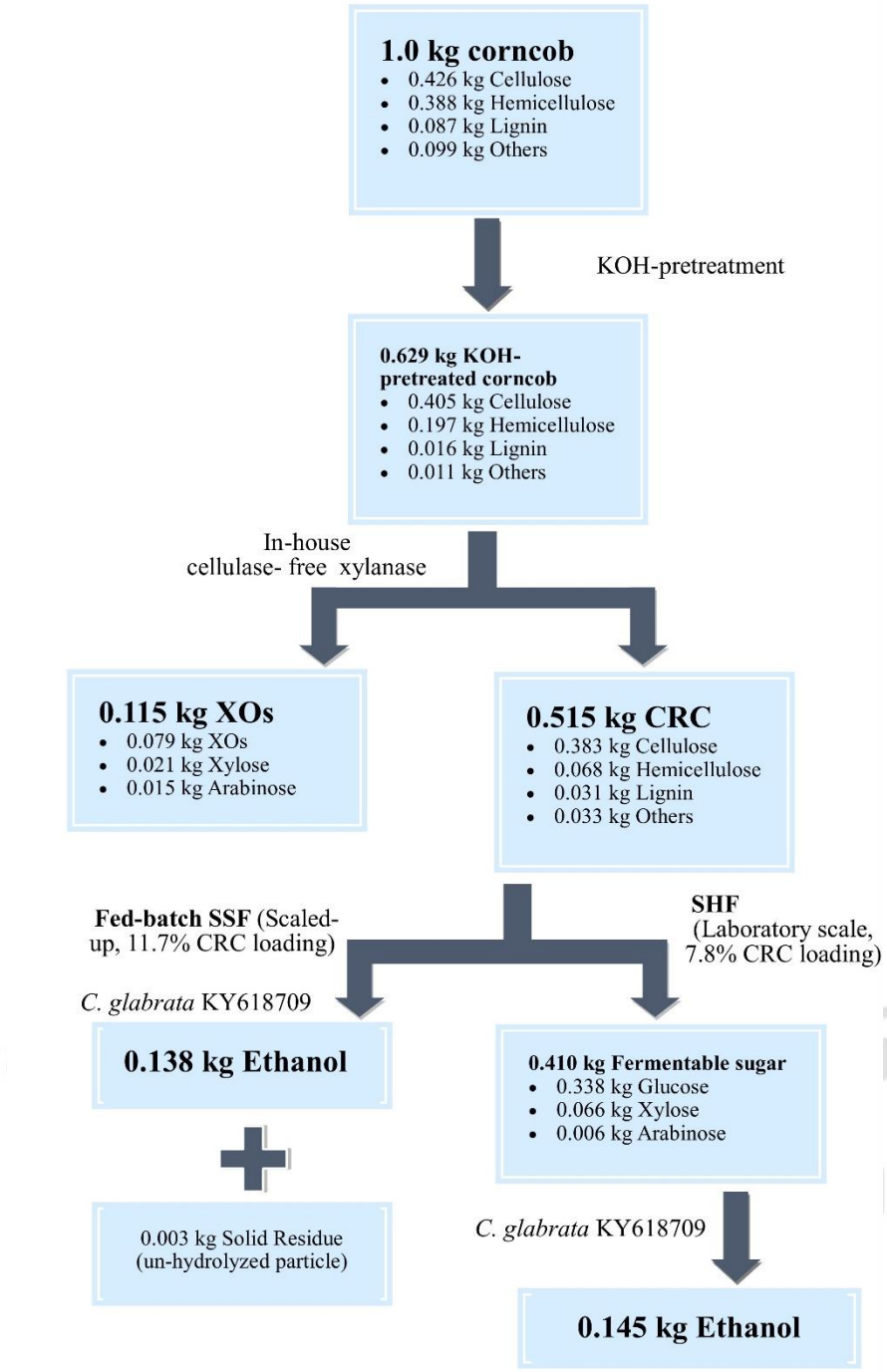

Figure 5.17 Mass balance of the integrated process for xylooligosaccharides and bioethanol production from corncob.

Nascimento Viviane et al. (2016) reported on XO and bioethanol production from sugarcane bagasse using an immobilized commercial xylanase. The XO concentration reached 12.44 g/L, while the ethanol concentration obtained via SHF with commercial *S. cerevisiae* was 19.41 g/L. Moreover, XOs and bioethanol can be produced by using wheat straw as substrate, though liquid hot water pretreatment (Huang et al., 2017). Commercial xylanase was applied in XO production whereas the fed-batch SSF was performed using commercial cellulase and commercial *S. cerevisiae*. XO and ethanol yields of 56.20 and 91.40 g, respectively, were obtained from 1 kg of wheat straw. Another integrated process has been reported by Zhu et al. (2013), who investigated an integrated process for ethanol, vanillin and XO production from *Camellia oleifera* (tea oil) shell at shaking-flask level. XOs and vanillin were produced by a commercial xylanase combined with a chemical method and ethanol was produced via SHF by commercial *S. cerevisiae*. The results showed relatively low concentrations of XOs, vanillin and ethanol: 1.76, 0.33 and 17.35 g/L, respectively. In comparison, promising concentration of 22.13 g/L (115 $g/kg_{raw\ corncob}$) of XOs and 31.32 g/L (138 $g/kg_{raw\ corncob}$) of ethanol were achieved by the integrated process in this study.

5.4 Conclusion

An integrated process to produce XOs and bioethanol via fed-batch SSF of corncob can be performed effectively and economically using an in-house thermostable xylanase from *S. thermovulgaris* TISTR1948 and newly isolated thermotolerant yeast *C. glabrata*. Corncob XOs had a high content of xylobiose (X2), the most desirable XOs in functional food application. Moreover, this integrated process generated 0.115 kg of XOs and 0.138 kg of ethanol from 1 kg corncob. It might be an alternative cost-effective approach to valorize corncob into high-value products using an in-house enzyme and fed-batch SSF which can be operated at elevated temperature with short processing time.