AVANCE 400 WB solid-state ^{13}C Nuclear Magnetic Resonance (NMR) spectrometer (Bruker, Germany) operating at a frequency of 100 MHz for carbon was used to obtain all spectra with cross-polarization (CP) and magic angle spinning (MAS). The spinning speed was 7 kHz, contact time was 2 ms, and pulse delay was 5 s. A total of 5000 scans were performed per sample at room temperature.

3. Results and discussion

3.1. Organosolv pretreatment

3.1.1. Organosolv pretreatment with three different types of catalysts

Pitch pine was treated at various conditions to select the appropriate condition for the pretreatment.

Digestibility of organosolv pretreated pitch pine with 1% sulfuric acid (SAE) increased when pretreatment temperature and residence time increased but decreased when conditions were extremely severe. The maximum digestibility was 53–57% at 160 °C for 20 min, 170 °C for 10 min, 170 °C for 20 min, 180 °C for 0 min, 180 °C for 10 min, and 190 °C for 0 min (Fig. 1a).

Enzymatic digestibility of organosolv pretreated materials with 1% magnesium chloride (w/v) (MCE) increased as temperature and residence time increased (Fig. 1b). This process showed its similar tendency with pretreatment using sulfuric acid. The maximum digestibility was 57–61% at 200 °C for 20 min, 210 °C for 10 min, and 210 °C for 20 min. Maximum digestibility was higher than that of pretreated material with sulfuric acid, but the organosolv process with magnesium chloride required much harsher condition than that with sulfuric acid.

Organosolv pretreatment with 1% sodium hydroxide (w/v) (SHE) did not improve the enzymatic digestibility of materials. Digestibility at all the conditions was only about 10%, which is similar to that of raw materials (data not shown).

To select the condition for the organosolv pretreatment with sulfuric acid and magnesium chloride, statistical analysis was conducted to confirm the statistical significance using the Statistical Analysis System (SAS, version 9.1) programming package. Each pretreatment condition was divided into several groups according to their digestibility (Table 1). A is labeled as the highest digestibility, and the succeeding order of alphabets is labeled for lower digestibility.

As stated in Table 1, in organosolv pretreatment with sulfuric acid, maximum digestibility groups ranges from A to B; therefore, groups A, AB, and B were selected. The organosolv process with magnesium chloride was grouped clearly; group A was selected. The harsher condition within the same group was eliminated. For example, organosolv pretreatment at 210 °C for 20 min with 1% magnesium chloride was not chosen for the process, but only pretreatment at 200 °C for 20 min and at 210 °C for 10 min was selected from group A.

3.1.2. Specific experiments for selecting optimal conditions

Pretreatment and enzymatic hydrolysis processes were repeated at selected conditions. For the organosolv process with 1% sulfuric acid, pretreatment at 160 °C for 20 min, 170 °C for 10 min, 170 °C for 20 min, 180 °C for 0 min, and 180 °C for 10 min was conducted. Pretreatment at 200 °C for 20 min and at 210 °C for 10 min was carried out for 1% magnesium chloride. Pretreated material yield (Araque et al., 2008), H-factor (Guillichsen

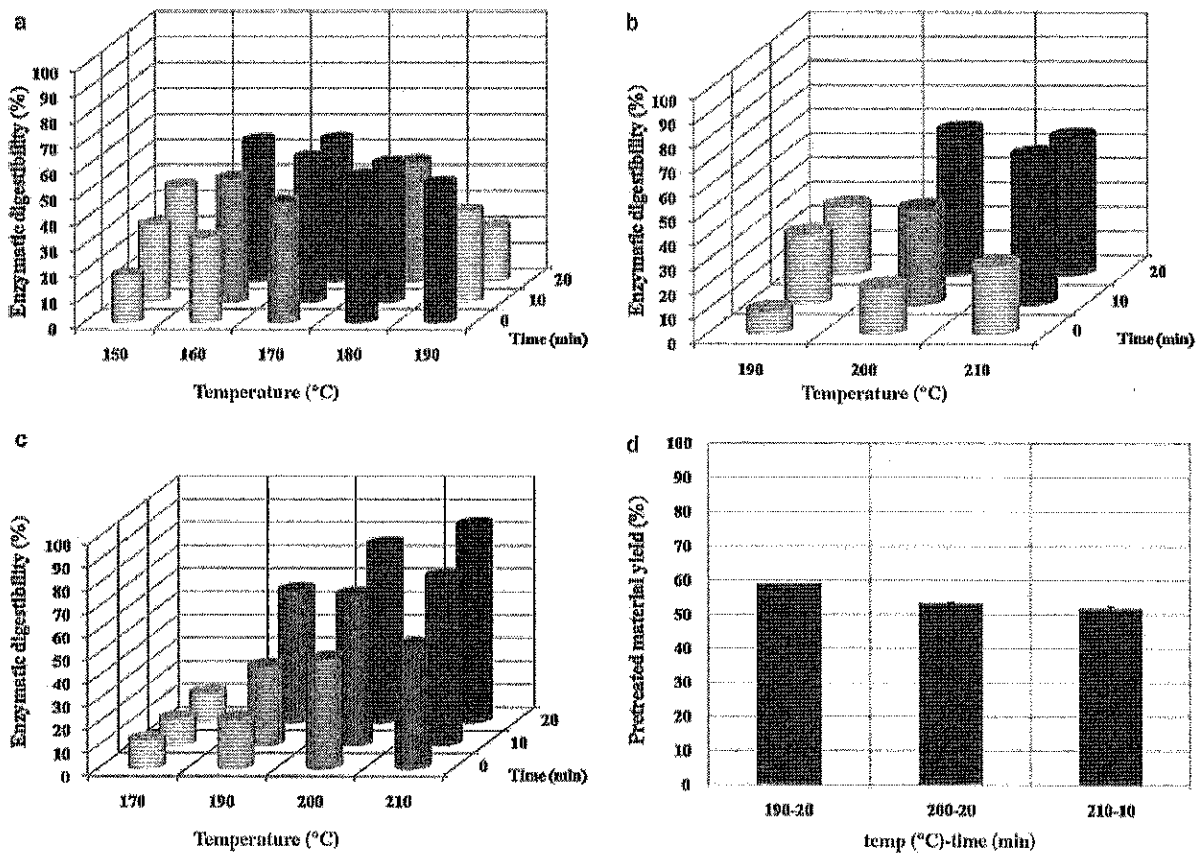

Fig. 1. Enzymatic digestibility of organosolv pretreated pitch pine with (a) 1% sulfuric acid and (b) 1% magnesium chloride. (c) Enzymatic digestibility, and (d) pretreated material yield of organosolv pretreated pitch pine with 2% sodium hydroxide.

Table 1
Grouping pretreatment conditions by SAS program according to the result of enzymatic digestibility.

Temperature	150 °C			160 °C			170 °C			180 °C			190 °C		
1% Sulfuric acid with 50% ethanol pretreatment															
Time	0	10	20	0	10	20	0	10	20	0	10	20	0	10	20
Group	I	G	E	F	C	AB	CD	AB	A	A	B	D	B	EF	H
Temperature		190 °C					200 °C					210 °C			
1% Magnesium chloride with 50% ethanol pretreatment															
Time		0		10		20	0		10	20			0	10	20
Group		E		D		C	D		B	A			C	A	A

Table 2
The experimental data of organosolv pretreatment process at selected conditions with three different catalysts.

Pretreatment temperature–time	Pretreated material yield (%)[c]	Digestibility (%)	Glucose yield (%)[d]	H-factor[e]	Severity factor (log *R*)[f]
SAE 160-20[a]	61.36 (±0.04)	54.99 (±1.98)	72.62 (±1.74)	173.6 (±4.6)	3.1
SAE 170-10	58.10 (±0.10)	56.25 (±1.81)	75.23 (±3.63)	231.0 (±4.0)	3.1
SAE 170-20	54.75 (±0.38)	55.50 (±1.23)	71.22 (±3.29)	366.9 (±15.5)	3.4
SAE 180-0	61.48 (±0.13)	56.99 (±1.92)	75.49 (±5.84)	154.4 (±13.4)	1.4
SAE 180-10	50.87 (±1.90)	53.87 (±1.93)	69.63 (±3.65)	480.0 (±39.7)	3.4
MCE 200-20[b]	67.66 (±0.30)	58.98 (±2.58)	74.49 (±2.94)	3481.5 (±77.6)	4.2
MCE 210-10	69.52 (±0.46)	61.17 (±1.63)	75.88 (±4.01)	3650.2 (±106.7)	4.2

[a] SAE, pretreatment with 1% sulfuric acid and 50% ethanol solution.
[b] MCE, pretreatment with 1% magnesium chloride and 50% ethanol solution.
[c] Pretreated material yield, mass (g) of pretreated material obtained from 100 g of dry woods.
[d] Glucose yield = 100 × (monomeric glucose (g)/oven-dried sample (g)).
[e] H-factor, a function of temperature and residence time including preheating time.
[f] Severity factor, a function of temperature and residence time without considering preheating time.

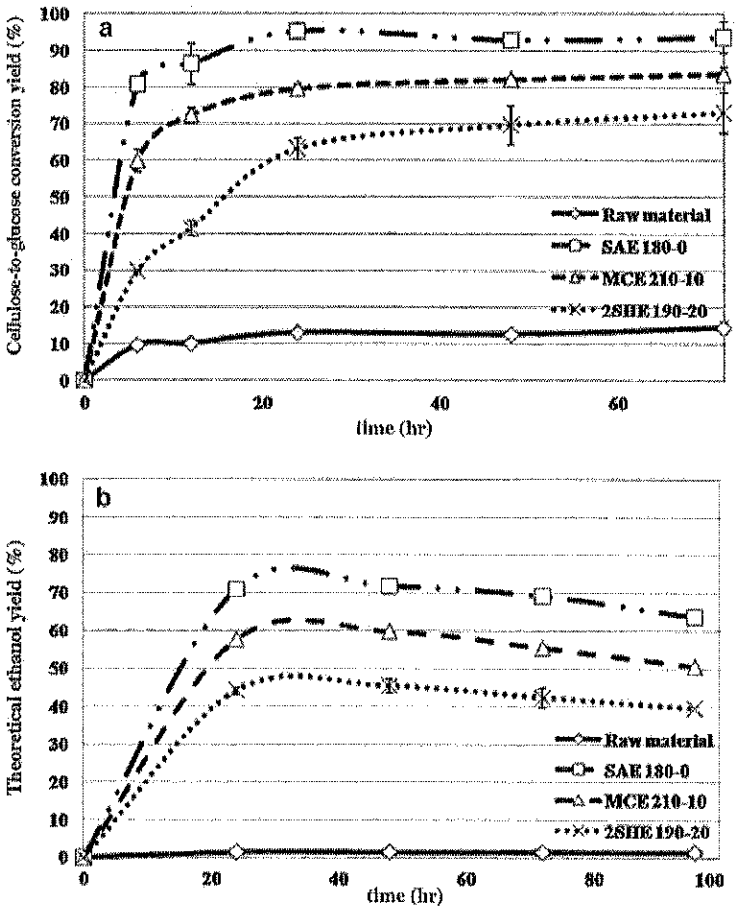

Fig. 2. (a) Cellulose-to-glucose conversion yield, and (b) theoretical ethanol yield of organosolv pretreated materials. SAE 180-0, organosolv pretreatment with 1% sulfuric acid at 180 °C for 0 min; MCE 210-0, organosolv pretreatment with 1% magnesium chloride at 210 °C for 10 min; 2% SHE 190-20, organosolv pretreatment with 2% sodium hydroxide at 190 °C for 20 min.