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Efficiencies of acid catalysts in the hydrolysis of lignocellulosic biomass over a range of combined severity factors

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ABSTRACT

Dicarboxylic organic acids have properties that differ from those of sulfuric acid during hydrolysis of lignocellulose. To investigate the effects of different acid catalysts on the hydrolysis and degradation of biomass compounds over a range of thermochemical pretreatments, maleic, oxalic and sulfuric acids were each used at the same combined severity factor (CSF) values during hydrolysis. Xylose and glucose concentrations in hydrolysates were highest with maleic acid. Oxalic acid gave the next highest followed by sulfuric acid. This ranking was particularly true at low CSF values. The concentrations of glucose and xylose increased with oxalic and sulfuric acid pretreatments as the CSF increased, but they never attained the levels observed with maleic acid. Among sulfuric, oxalic and maleic acid treatments, the amount of xylose released as xylooligosaccharide was highest with sulfuric acid. The fraction of xylooligosaccharide was lowest with the maleic acid and the oligosaccharide fraction with oxalic acid fell in between. Furfural and hydroxymethyl furfural levels were also highest with maleic acid. In subsequent fermentations with pretreated biomass, the ethanol concentration was maximal at 19.2 g/l at CSF 1.9 when maleic acid was used as the pretreatment catalyst. This corresponded to an ethanol volumetric production rate of 0.27 g ethanol/l per h. This was the same condition showing the highest xylose production in following pretreatment with various acid catalysts. These findings suggest that maleic and oxalic dicarboxylic acids degrade hemicelluloses more efficiently than does sulfuric acid.

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1. Introduction

Growing worldwide attention has been focused on the use of lignocellulosic biomass for fuel ethanol production in order to mitigate competition with food supplies while reducing release of fossil carbon (Bartle and Abadi, 2010; Tilman et al., 2009). The hemicelluloses and cellulose in lignocellulosic biomass require pretreatment to hydrolyze the polymers to fermentable sugars or oligosaccharides prior to fermentation. Pretreatment is essential for enzymatic or acid saccharification because native lignocellulose is highly recalcitrant due to lack of porosity, the presence of side chains, the crystallinity of cellulose and the high molecular weights and impermeable nature of the heterogeneous matrix.

A pretreatment should fulfill the following conditions: (1) produce substrates with high cellulose content and susceptibility towards enzymatic hydrolysis, (2) limit production of undesired degradation products such as phenolic compounds, furfural or 5-hydroxymethylfurfural (HMF), and (3) allow high recovery of valuable hemicelluloses-derived products. For the purposes of pretreatment, various methods such as steam explosion, steam explosion with dilute sulfuric acid (Grohmann et al., 1985; Torget et al., 1991; Lloyd and Wyman, 2005), organosolv extraction (Pan et al., 2005), and biological treatment with white rot fungi (Ferraz et al., 2001) have been extensively investigated. Recently, supercritical and subcritical water treatments have been of great interest because of their potential to induce the direct conversion of biomass resources into sugar without a saccharification step (Sasaki et al., 1998; Matsumura et al., 2005).

Chemical pretreatment with sulfuric acid has been the traditional method to hydrolyze lignocellulosic biomass. However, it suffers several disadvantages, such as the production of inhibitory products and the corrosion of equipment. In order to overcome these disadvantages, dicarboxylic acids such as maleic and oxalic acids have been suggested as alternatives for sulfuric acid in the pretreatment (Kootstra et al., 2009; Lu and Mosier, 2007; Lee et al., 2009). Maleic and oxalic acids have higher pKa values than sulfuric acid and thus a higher solution pH, compared to mineral acids such as sulfuric acid. Since they are dicarboxylic organic acids,

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they also exhibit two pKa values, which may impart more efficient hydrolysis of the substrate over a range of temperature and pH values. In particular, maleic acid shows an especially high k_{hyd}/k_{deg} ratio, which favors cellulose hydrolysis to glucose over glucose degradation (Mosier et al., 2002). Additionally, oxalic acid is less toxic to yeasts and other microbes than acetic or sulfurous acids, does not inhibit glycolysis and does not produce noxious odors (Mosier et al., 2001; Kootstra et al., 2009). However, a comparison of maleic and oxalic acids with sulfuric acid in the degradation of lignocellulosic biomass has not previously been investigated. In the present study, we examined the degradation of hemicelluloses by these three acid catalysts during pretreatment over a range of acid concentrations while keeping the combined severity factor (CSF) constant. The CSF is an index that integrates changes in temperature, time and acidity into a single value, which facilitates comparisons of different conditions (Abatzoglou et al., 1992). By keeping the CSF constant over a range of treatments with different acids, we hypothesized that the catalytic rates and product yields would reveal properties specifically attributable to the dicarboxylic properties of the organic acids. The findings showed highly significant differences among the three acids, with the dicarboxylic acids exhibiting much higher hydrolytic efficiencies. We then assessed the utility of the hydrolysates for ethanol production in order to reveal any differences acid catalysts during pretreatment of biomass.

2. Methods

2.1. Biomass and pretreatment condition

Standardized corncob pellets (Pestell, New Hamburg, Canada) with a moisture content of 10% (w/w) were used in this study. The pellets are globular with a diameter of ca. 0.5 mm. The pretreatment process was based on the method developed by Kenealy et al. (2007). Corncob and acid solutions were placed in stainless steel pressure vessels (64 mm diameter × 360 mm, approximately 1100 cc volume) that were in turn put into a larger tumbling digester, steam heated to the reaction temperature, and then rotated to keep the liquor in contact with the material during cooking. Each vessel was loaded with 75 g (dry weight basis) of material and sufficient acid/water mixture to give a total solid/liquid ratio of 1:4 (w/w). To investigate the effect of various acids during pretreatment, the reaction temperature and time were fixed at each CSF. The pretreatment condition is shown in Table 1. The liquid fraction (hydrolysate) was separated by vacuum filtration from the pretreated biomass after pretreatment and stored at 4 °C for sugar and inhibitors analysis. The pretreated biomass was washed with 300 ml of water then used as a substrate for simultaneous saccharification and fermentation (SSF).

2.2. Combined severity factor (CSF)

For the pretreatment, a calculated CSF was used to integrate the effects of reaction times, temperature and acid concentration into a single variable (Lloyd and Wyman, 2005). However, the pH was the only factor affecting the pretreatment in this study. The CSF used in this study is defined as

$$\mathrm{CSF} = \log\{t \cdot \exp[(T_H - T_R)/14.75]\} - \mathrm{pH} \quad (1)$$

where t is the reaction time of the pretreatment in minutes, T_H the reaction temperature in °C, T_R the reference temperature, most often 100 °C, and pH is the acidity of the aqueous solution in terms of acid concentration. Acid loadings were based on pH value.

Table 1
pH and acid concentrations used for each calculated severity factor (CSF)[a].

CSF	pH[b]	Acid (g/l)		
		Sulfuric	Oxalic	Maleic
1.8	1.5	1.67	3.67	5.33
1.9	1.4	3.00	5.00	9.10
2.0	1.3	4.67	6.67	11.67
2.1	1.2	6.67	8.67	15.33

[a] All reaction conditions used 170 °C for 18 min.
[b] Acid added to adjust pH as theoretical and then confirmed the pH value during making acid solution with pH meter.

2.3. Chemical analysis of pretreated biomass

The carbohydrate compositions of the pretreated corncob were determined according to the method of Davis (1998) using a high-performance anion exchange chromatograph (ICS-3000, Dionex, Sunnyvale, CA) with pulsed amperometric detection (HPAEC-PAD). Samples were milled to pass a 1 mm screen using a Wiley mill (Thomas Scientific, Swedesboro, New Jersey) and dried at 45 °C. Pretreated corncob was hydrolyzed with 72% (w/w) H_2SO_4 for 1 h at 30 °C. Hydrolysates were brought to a final H_2SO_4 concentration of 4% (w/w) and then heated at 120 °C for 1 h. After hydrolysis, 2 ml of supernatant was centrifuged, filtered through a 0.45 μm porous membrane, and analyzed.

2.4. Sugar and inhibitors analysis

The concentrations of glucose, xylose, arabinose and acetic acid in the hydrolysate were determined using HPLC (Gilson 307 system, Middleton, WI) outfitted with an Aminex 300 × 7.8 mm column (Bio-Rad, Hercules, CA) and a refractive index detector (Hitachi High Technologies Corporation model L-2490, Japan). Analysis was performed with 5 mM H_2SO_4 as the mobile phase at 0.3 ml/min for 55 min and the analysis was done under isocratic mode. HMF and furfural in the hydrolysate were measured by HPLC (HP, 1090 Series II, Hewlett–Packard, Now Agilent Technologies, Palo Alto, CA) with a Phenomenex C18(2) column (250 × 4.6 mm) and acetonitrile, water and 1% acetic acid as the mobile phase at a flow rate of 0.8 ml/min. All samples were properly diluted and filtered through a 0.45 μm spin-filter before analysis to remove the particle. Total phenolic compounds (TPCs) were estimated colorimetrically by the Folin–Ciocalteu method via a standard curve relating 760 nm absorbance (Scalbert et al., 1989). All the analyses were carried out in triplicate.

2.5. Oligomer sugar analysis in hydrolysate

Oligomer sugar analysis was based on NREL's Laboratory Analytical Procedure: "Determination of Sugars, Byproducts, and Degradation Products in Liquid Fraction Process Samples" (Jensen et al., 2010). The filtered samples of hydrolysate were brought to a final H_2SO_4 concentration of 4% (w/w), autoclaved at 121 °C for 1 h, and then centrifuged for HPLC determination of the monosaccharide hydrolyzed from oligomer (as described in Section 2.4).

2.6. Yeast strain

Scheffersomyces (*Pichia*) *stipitis* CBS 6054 (Kutzman and Suzuki, 2010) was used for SSF of the solid fraction. The strain was maintained on agar malt medium. Cells were grown in 1000 ml Erlenmeyer flasks containing 400 ml of YPD (10 g/l yeast extract, 10 g/l peptone, 20 g/l glucose) in an orbital shaker incubator at 30 °C, shaken at 200 rpm. Following 24 h growth, cells were harvested by centrifugation (6708g, 10 min, 4 °C), washed with sterile DI water and adjusted to a calculated concentration of 30 g/l dry cell weight (DCW) via standard curves relating 600 nm

absorbance to DCW/l concentration. The initial cell concentration was 2.0 DCW/l for SSF.

2.7. Simultaneous saccharification and fermentation (SSF)

The pretreated corncob (5 g dry wt.) was transferred to a 125 ml Erlenmeyer flask containing 50 ml of fermentation medium, which consisted of 5 g/l yeast extract, 5 g/l urea, 0.5 g/l $MgSO_4 \cdot 7H_2O$ and 1 g/l KH_2PO_4 in 50 mM sodium citrate buffer. For saccharification, cellulase (500 CMC U/g dry wt. biomass) and β-glucosidase (80 pNPG U/g dry wt. biomass) of Acellerase 1000 from Genecor (Danisco, Rochester, NY) were added to the fermentation medium. SSF was started by adding enzymes and *Scheffersomyces stipitis* to the samples, which were then incubated at 30 °C in an orbital shaker at 150 rpm. Samples were taken at 24, 48, 72 h, and the amounts of monosaccharides remaining and ethanol produced were analyzed by HPLC (as described in Section 2.4). All determinations were performed in triplicate biological replications.

3. Results and discussion

3.1. Sugar and inhibitors in hydrolysate by acid pretreatment

We investigated the different catalytic properties of maleic, oxalic and sulfuric acids on the degradation of the biomass compounds over a range of CSF values. The monosaccharide concentrations contained in the hydrolysate by acid pretreatment of corncob are shown in Fig. 1. Xylose was the most abundant sugar in the hydrolysate subjected to acid catalyst pretreatment. When all three acid catalysts were compared at the same CSF, the xylose concentrations differed significantly, which agrees with the report of Guo et al. (2009). The xylose concentration in the maleic acid hydrolysate was particularly high. The xylose concentration in maleic hydrolysates dramatically increased as the CSF was raised from 1.8 to 1.9 and then slowly increased as the CSF was further raised above 2.1. The latter, slower increase was accompanied by the degradation of xylose into byproducts, mainly furfural (Fig. 3). By comparison, when oxalic and sulfuric acids were used, the xylose concentration increased significantly as the CSF was raised from 2.0 to 2.1. As shown in Fig. 1b, the glucose obtained from the acid pretreatment gradually increased with increasing severity, although the yields were low compared with xylose. A similar trend was previously reported for the lignocellulosic biomass pretreated with acid catalyst (Jensen et al., 2010; Lee et al., 2010; Pederson and Meyer, 2010).

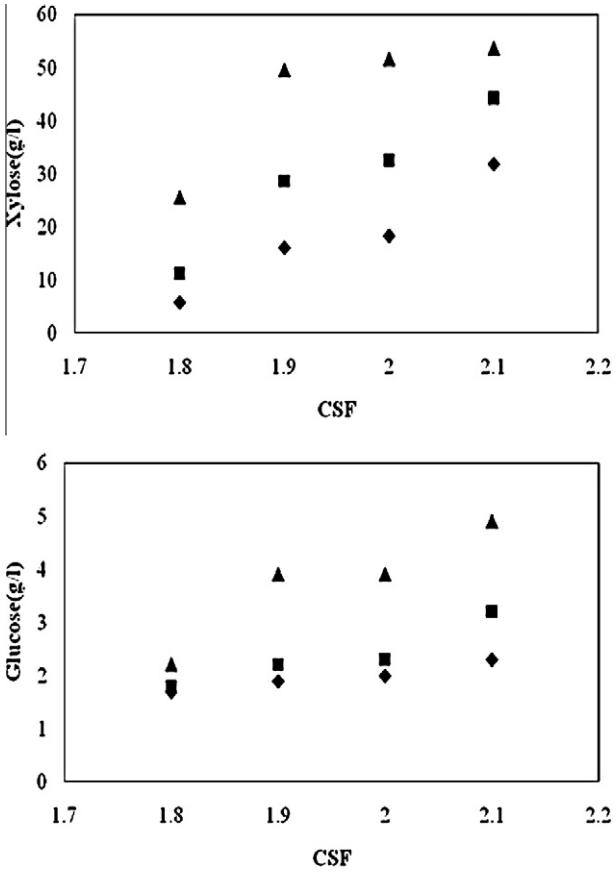

Fig. 1. Concentration of glucose and xylose obtained in hydrolysates after pretreatment by various acid catalysts over a range of combined severity factors (CSF) (♦: sulfuric acid, ■: oxalic acid, ▲: maleic acid).

A two-stage process is available for the high production of glucose and xylose from lignocellulosic biomass (Rodriguez-Chong et al., 2004). In the first stage, the hemicellulose is hydrolyzed at a lower temperature and in the second stage the cellulose in the residual solids is hydrolyzed at a higher temperature. Therefore, the pretreatment conditions applied in this study provided elevated xylose with minimal glucose from the first stage hydrolysis. However, the condition for maximizing the xylose production was not consistent among the three acid catalysts, as the maleic and oxalic acid pretreatments produced higher xylose than the sulfuric acid pretreatment. Oxalic and maleic acids are among the strongest organic acids known, and their dicarboxylic properties are thought to yield superior selectivity for the hydrolysis of hemicelluloses (Mosier et al., 2002; Lu and Mosier, 2007; Lee et al., 2010).

The concentrations of xylose, glucose and arabinose released in the prehydrolysis and the total amounts of sugars and acetic acid released following post-hydrolysis with 4% sulfuric acid, are shown in Fig. 2. The differences between pre- and post-hydrolysis sugar concentrations were used to estimate the amount of xylooligosaccharide solubilized in the prehydrolysis stage. Following pretreatment at low CSF (1.8), the xylooligosaccharide concentration was high when maleic, oxalic and sulfuric acids were used as catalysts, however, all three acids produced more monosaccharide and less xylooligosaccharides as the CSF increased. Following pretreatment at moderate CSF (2.0) all of the xylose was present as monomer rather than as xylooligosaccharide in the maleic acid hydrolysate, whereas at the same severity, both oxalic and sulfuric acid hydrolysates still contained significant amount of oligosaccharide. Following pretreatment at successively higher severity, the concentration of xylose increased with the CSF in the sulfuric acid and oxalic acid pretreatments. As evident from the furfural and HMF analyses, xylose liberated from the maleic acid pretreatment began to degrade to furfural above CSF 1.8 and the concentration of furfural and HMF in the maleic acid pretreatment reached a plateau at CSF 1.9 or above (Fig. 3). For the sulfuric and oxalic acid hydrolysates, furfural and HMF production increased as more xylose and glucose monomer became available for degradation.

The yields of total sugars (xylose and glucose:monomer plus oligomer) were maximal at a CSF between 1.9 and 2.1, and the effect of increasing CSF over 2.1 was insignificant (data not shown). The solubility of biomass components by acid pretreatment was very similar, although the type of degraded product of biomass varied greatly among the three acid catalysts even as the H^+ concentration remained the same. This result was attributed to the ability of biomimetic catalysts such as dicarboxylic acids to aid the hydrolysis of hemicellulose (Shimada and Takahashi, 1991). Enzymes for degrading cellulose and hemicellulose catalyze hydrolysis through a general acid–base mechanism mediated by two carboxylic acid moieties on the side chains of the amino acids

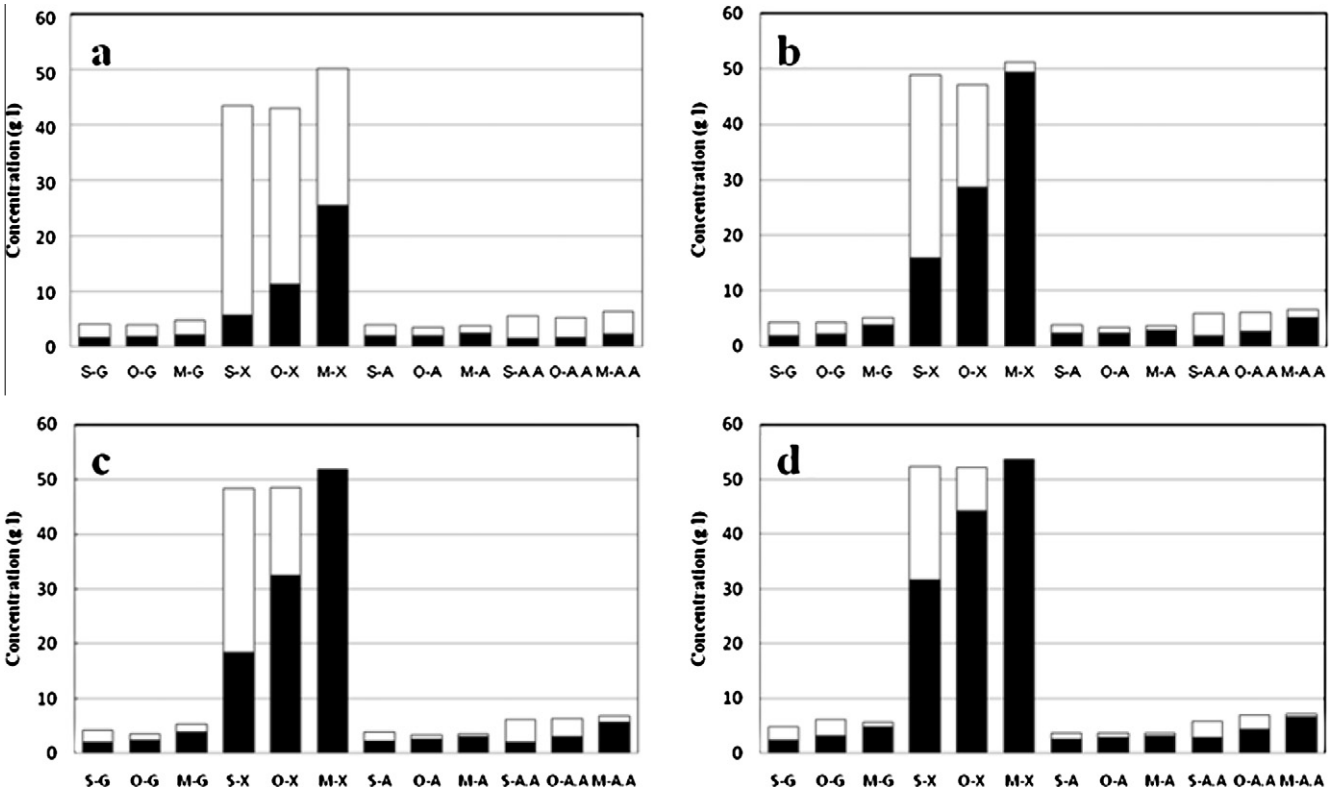

Fig. 2. Monomeric sugars and acetic acid concentrations released from oligosaccharide the in hydrolysate according to the combined severity factor (a: CSF 1.8, b: CSF 1.9, c: CSF 2.0, d: CSF 2.1, S: sulfuric acid, O: oxalic acid, M: maleic acid, G: glucose, X: xylose, A: arabinose, A.A: acetic acid). Total sugar or acid content as determined by 4% sulfuric acid post-hydrolysis is indicated by the open bars; sugar or acid released from pretreatment is indicated by the closed bar.

present in the enzyme active site, which is mimicked by dicarboxylic acid (Breslow, 1995; Lawson et al., 1997).

The oligomers in the hydrolysate should be considered since they could not easily be fermented to ethanol by microorganisms such as *Saccharomyces*. Therefore, extra enzymes are needed to degrade the oligomer to monosaccharide for ethanol production during the fermentation step. Xylooligosaccharides also have recently been shown to inhibit enzymes greatly (Kumar and Wyman, 2009). The amounts of glucose and arabinose as oligosaccharides were low (Fig. 2) and did not differ significantly according to the kind of acid catalyst. Acetic acid is generated by the hydrolysis of the acetyl groups on hemicelluloses and is commonly observed along with the release of xylose. Therefore, the acetic acid concentration increased in proportion to the oligomer concentration (Kabel et al., 2002).

Fig. 3 shows the concentration of the fermentative inhibitors HMF and furfural at various CSF levels. Furfural is generated by conversion from xylose under severe pretreatment conditions. A furfural concentration of 833 mg/l was generated at a CSF of 2.1, which had the highest xylose yield when maleic acid was used as the pretreatment catalyst. The trend of furfural and xylose were very similar over the CSF range studied. In contrast, the HMF concentration was very low, even at the highest CSF, which indicated that the acid catalyst had little effect on cellulose hydrolysis or glucose degradation. The HMF concentration ranged from 58 to 208 mg/l at the lowest and highest CSF, respectively. The HMF concentration induced from glucose was proportional to glucose produced during pretreatment and it depended on CSF (Kim et al., 2011).

TPCs (total phenolic compounds) are byproducts derived from lignin by acid hydrolysis that inhibit the growth and ethanol production of fermentative microbes. The amounts of TPCs dissolved in hydrolysates did not appear to differ among the three acid catalysts. We did not characterize the natures of the lignin degradation products, but the total amounts of TCPs as determined by our analysis did not differ under the conditions examined. This indicated that lignin degradation was not significantly affected by the kind of acid catalyst or the severity condition for pretreatment.

3.2. Chemical composition of the residual biomass following pretreatment

Composition of the residual biomass that was left after hydrolysis depended on the CSF and acid employed (Fig. 4). Our analysis showed the raw material to contain 13.9% lignin, 2.19% arabinan, 37.9% glucan and 27.83% xylan prior to hydrolysis, which agreed with the report of Barl et al. (1991). Among the carbohydrates, the arabinoxylans content was higher than that of other agricultural wastes (Makishima et al., 2009). The carbohydrate contents of the residual biomass ranged between 59.01% and 69.12%. This is reflected in the higher lignin content of the residual solids, which ranged between 24% and 32%. The hemicellulose contents showed a closely related pattern, as they decreased steadily with the severity of the treatments and ranged between 4% and 16% of the residual dry wt. On the other hand, the rate of cellulose degradation was significantly different from that of hemicellulose, as it did not exhibit any significant dependence on CSF or the kind of acid catalyst. The amount of glucose found in the liquid fraction corresponded to less than 1% of that in the original raw material, which indicated that the cellulose was not easily degraded to fermentable sugar by these acid catalysts under the condition employed. We considered whether partial delignification of the pretreated material could have increased the overall glucan yield and phenolic

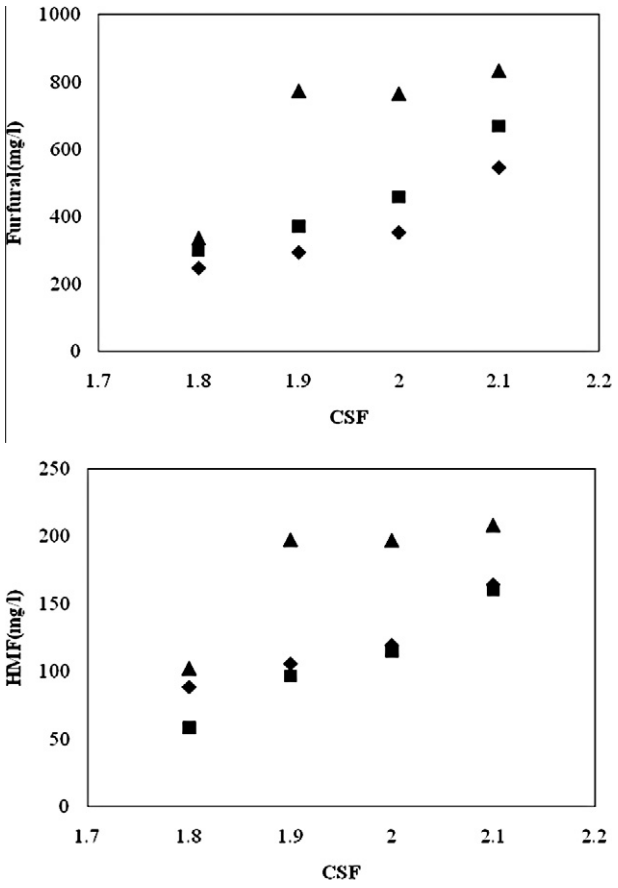

Fig. 3. Comparison of inhibitors generated from the biomass after different acid catalyst pretreatment (♦: sulfuric acid, ■: oxalic acid, ▲: maleic acid).

compound content in the liquid fraction. However, the lignin content of the pretreated corncob, representing between 23.8% and 37.5% of the pretreated material as compared to 13.9% of the raw material, increased concomitantly with the pretreatment condition due to hemicellulose loss. This trend has been previously reported on the lignocellulosic biomass pretreated with dilute sulfuric acid (Ohgren et al., 2007; Cara et al., 2008; Kumar et al., 2009).

3.3. Simultaneous saccharification and fermentation (SSF)

Ethanol yield obtained depended on the pretreatment condition, as shown in Table 2. After 72 h fermentation, glucose and xylose were converted into ethanol across the range of CSF studied. The ethanol concentration increased rapidly in the first 24 h because of the high initial rate of enzymatic hydrolysis during SSF, and the process was completed after 96 h (data not shown). However, the ethanol yield (Q_p) was highest in the first 72 h. Ethanol production peaked at 19.2 g/l with maleic acid at CSF 1.9. This corresponded to an ethanol volumetric production rate of 0.27 g ethanol/l h. This condition also attained the highest xylose production during pretreatment. Interestingly, each pretreated biomass showed a different ethanol production after SSF, despite the use of the same pretreatment with the same CSF for each acid catalyst. This indicated that the biomass structure for enzymatic hydrolysis was affected by the kind of acid catalyst. This was confirmed by glucose production during enzymatic hydrolysis, which increased with treatment severity when sulfuric acid was used as the pretreatment catalyst. In contrast, glucose production from biomass pretreated with oxalic and maleic acids were highest at CSF 2.0 and 1.9, respectively. Glucose production by enzymatic hydrolysis of the pretreated biomass was closely connected with hemicelluloses degradation during pretreatment. One significant beneficial effect of hemicellulose hydrolysis is the destruction of the protective sheath from around the cellulose. Its removal enhances the accessibility of cellulose to cellulose and increases enzymes effectiveness (Jeoh et al., 2007).

Overall, the experimental ethanol yields when oxalic and maleic acids were used as the pretreatment catalyst were higher than those obtained with sulfuric acid, even though the sulfuric acid catalyst showed a high experimental ethanol yield at CSF 1.8. Otherwise, the ethanol production was only 11.4 g/l due to the low efficiency of enzymatic hydrolysis of the biomass pretreated with sulfuric acid. Ethanol production, the ethanol yield and Q_p were all maximized at CSF 1.9 with maleic acid. These results suggest that maleic catalyst can improve the sugar yield of enzymatic hydrolysis and the ethanol production of SSF in the low CSF compared with that of sulfuric acid. To maximize the ethanol production, the catalytic pretreatment using maleic acid required low CSF while that using sulfuric acid needed high CSF. This difference was attributed to the divergence in the activation energy for xylose degradation between maleic and sulfuric acids, as reported by Lu and Mosier (2007). Although the maleic acid cost per unit of biomass is an order of magnitude greater than that of sulfuric acid, one can infer from the higher yields using maleic acid that the use of a dicaboxylic acid can increase the efficiency of hydrolysis. Even though the ethanol yields were lower with oxalic acid pretreatment than with maleic pretreatments, the substantially lower cost of could offer the potential to save energy and enzyme for ethanol production.

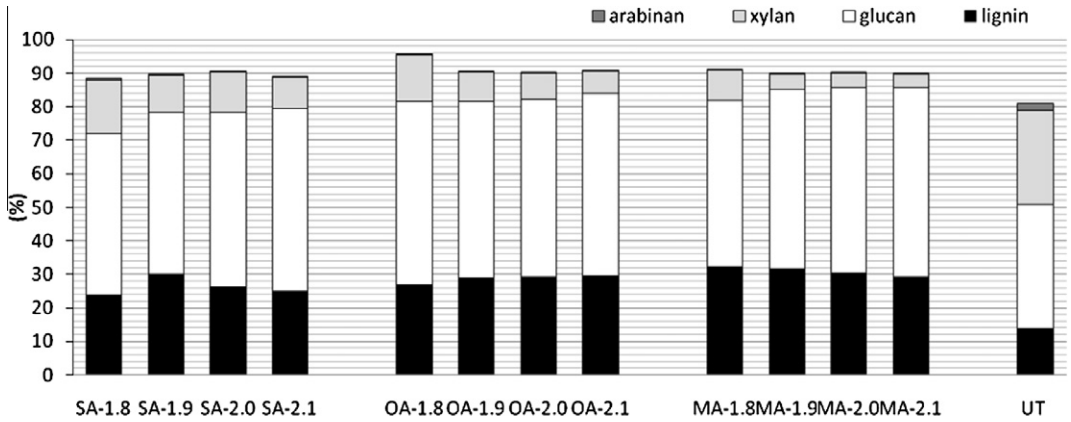

Fig. 4. Composition of residual biomass following pretreatment with the different acid catalysts (SA: sulfuric acid, OA: oxalic acid, MA: maleic acid, UT: untreated corncob).

Table 2
Concentrations of sugars and ethanol accumulated or remaining following simultaneous saccharification and fermentation (SSF) of residual solids.

Pretreatment condition	Glucose (g/l)		Xylose (g/l)		Ethanol (g/l)	Experimental ethanol yield[b]	Q_p[c] (g/l/h)
	Following SSF	Without SSF	Following SSF	Without SSF	Following SSF		
SA-1.8	0	15.0[a]	0	5.1	11.4	0.57	0.16
SA-1.9	0	21.5	0	6.5	13.6	0.49	0.19
SA-2.0	0	24.6	0	7.3	14.5	0.45	0.20
SA-2.1	0	27.6	0.1	7.7	15.2	0.43	0.21
OA-1.8	0	20.7	0	6.0	12.0	0.45	0.17
OA-1.9	0	27.1	0.2	7.7	16.5	0.48	0.23
OA-2.0	0.1	28.8	0.3	8.2	16.7	0.46	0.23
OA-2.1	0.1	27.3	0.2	7.5	16.9	0.49	0.23
MA-1.8	0	21.5	0.1	6.5	15.7	0.50	0.22
MA-1.9	0.2	26.1	0.3	7.6	19.2	0.58	0.27
MA-2.0	0.3	25.3	0.3	7.6	18.5	0.57	0.26
MA-2.1	0.2	23.8	0.3	7.4	17.6	0.57	0.24

[a] Values in parentheses are at 30 °C, 150 rpm for 48 h following enzymatic saccharification but without the yeast strain.
[b] Experimental ethanol yield was calculated as ethanol production/(total sugar-remaining sugar).
[c] Q_p = ethanol volumetric productivity.

4. Conclusion

This work investigated the effect of different acid catalysts on the hydrolysis and degradation of biomass compounds when each was used at the same combined severity factor (CSF) values during pretreatment. Then it evaluated ethanol production with pretreated biomass from that condition. Sulfuric, oxalic and maleic acid were used as acid catalysts. Maleic and oxalic acid can release more sugars in the hydrolysate and more ethanol can be produced from the residual solids than can be achieved with sulfuric acid when each is used at the same CSF. Dicarboxylic acids such as maleic and oxalic acid produced more monomer sugar than oligomer and left behind a larger residual fraction of cellulose which implies that the dicarboxylic acid is more selective for hemicellulose.

Acknowledgements

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