



Differential effects of mineral and organic acids on the kinetics of arabinose degradation under lignocellulose pretreatment conditions

A. Maarten J. Kootstra^{a,c,*}, Nathan S. Mosier^b, Elinor L. Scott^a, Hendrik H. Beftink^c, Johan P.M. Sanders^a

^a Valorisation of Plant Production Chains, Wageningen University, P.O. Box 17, 6700 AA Wageningen, The Netherlands

^b Laboratory of Renewable Resource Engineering, 500 Central Drive, Purdue University, West Lafayette, IN 47907, USA

^c Food and Bioprocess Engineering Group, Wageningen University, P.O. Box 8129, 6700 EV Wageningen, The Netherlands

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ABSTRACT

Sugar degradation occurs during acid-catalyzed pretreatment of lignocellulosic biomass at elevated temperatures, resulting in degradation products that inhibit microbial fermentation in the ethanol production process. Arabinose, the second most abundant pentose in grasses like corn stover and wheat straw, degrades into furfural. This paper focuses on the first-order rate constants of arabinose (5 g/L) degradation to furfural at 150 and 170 °C in the presence of sulfuric, fumaric, and maleic acid and water alone. The calculated degradation rate constants (k_d) showed a correlation with the acid dissociation constant (pK_a), meaning that the stronger the acid, the higher the arabinose degradation rate. However, de-ionized water alone showed a catalytic power exceeding that of 50 mM fumaric acid and equaling that of 50 mM maleic acid. This cannot be explained by specific acid catalysis and the shift in pK_w of water at elevated temperatures. These results suggest application of maleic and fumaric acid in the pretreatment of lignocellulosic plant biomass may be preferred over sulfuric acid. Lastly, the degradation rate constants found in this study suggest that arabinose is somewhat more stable than its stereoisomer xylose under the tested conditions.

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1. Introduction

Future oil shortages, increasing oil prices and international agreements are reasons for increased research on alternative routes to produce chemicals and transportation fuels. Fermentation technology can produce such liquid fuels, but the feedstock (fermentable sugars) and processing costs need to be sufficiently low to compete economically with oil-derived fuels. In current first generation bioethanol production, relatively expensive sugar and starch derived from sugar cane and maize are used as feedstock. However, second generation processes will use relatively cheap and more abundant renewable lignocellulosic raw material, such as agricultural residues like corn stover, wheat straw, or forestry by-products. Using these by-product streams also results in less competition for high-quality edible carbohydrates.

Lignocellulosic biomass requires pretreatment to facilitate the hydrolysis of cell wall polysaccharides to fermentable sugars [1].

Pretreatment usually combines a catalyst (acid or base) in water with thermal treatment. For example, sulfuric acid pretreatment is used at 50–300 mM at 100–200 °C to hydrolyze hemicellulose, disrupt lignin, and render the residual cellulose more reactive when exposed to cellulolytic enzymes [1–4]. During the acid pretreatment at elevated temperature, degradation of the fermentable sugars occurs. Degradation products like furfural from pentoses and 5-hydroxymethylfurfural (HMF) from hexoses are inhibitory to yeasts in subsequent sugar-to-ethanol fermentation processes, which results in a lower efficiency of the ethanol production process [5–8].

At elevated temperatures, furfural degrades further into formic acid [9], while HMF degrades into both formic and levulinic acid [5,6]. In warm season grasses like wheat and maize, the hemicellulose fraction of the structural polysaccharides largely consists of arabinoxylan or glucuronoarabinoxylan (GAX) [10–12]. Thus, arabinose is the second most abundant pentose present in biomass like corn stover and wheat straw. While lignocellulosic materials contain much less L-arabinose than D-xylose, the relative amounts of the sugars strongly depend on the raw material. For example, on a dry matter basis corn stover contains 15% xylan and 3% arabinan, wheat straw contains 19% xylan and 2% arabinan, whereas wheat bran contains 19% xylan and 15% arabinan

* Corresponding author at: Valorisation of Plant Production Chains, Wageningen University, P.O. Box 17, 6700 AA Wageningen, The Netherlands. Tel.: +31 317 481315; fax: +31 317 483011.

E-mail address: maarten.kootstra@wur.nl (A.M.J. Kootstra).

[13,14]. Priority has been given to efforts to develop metabolically engineered microbes to ferment xylose to ethanol. However, recent efforts have been initiated to develop microbes able to convert arabinose to ethanol in order to increase yields proportionally [15–18]. In addition, arabinose is a pentose and, like xylose, can be degraded to furfural [9,19–21]. If the degradation rate of arabinose is similar to or higher than that of xylose (or glucose), its presence and behavior during the pretreatment may have an important negative influence on the ethanol production process. Since feedstock constitutes a substantial fraction of the end product prices, improving yield is important to the economic success of commodity chemical and fuel production. While arabinose may not be the most important sugar defining ethanol yield, its significance cannot be overlooked in the development of lignocellulose conversion technologies.

Maleic acid has been described as a possible alternative to sulfuric acid in acid pretreatment [22], resulting in high glucose yields and in lower amounts of inhibitory by-products. The latter is explained by the fact that while sulfuric acid is strong, maleic acid is a weak acid and sugar degradation is acid-catalyzed [19,21,23,24]. In addition, xylose degradation has been shown to be much slower in the presence of maleic acid compared with sulfuric acid below 175 °C [25].

Application of sulfuric acid also leads to a large inorganic waste stream, mostly gypsum. Using organic acids in the pretreatment would increase the quality of the by-product stream. An organic by-product stream would logically be more easily applied in co-firing installations, in fertilizing soil, and in animal feed [26,27]. Thus there is interest in using organic acid to pretreat lignocellulosic biomass, including maleic, succinic, and acetic acid [22]. Fumaric acid is similar in structure to maleic acid (*trans*- and *cis*-butenedioic acid, respectively) and is stronger than succinic acid. Fumaric acid may be produced *in situ* by fermentation, and together with acid recycling [28–30] these are possible options to further improve the efficiency of the whole ethanol production process.

In acid pretreatment of lignocellulose, the dilemma is that intensifying the acid pretreatment conditions to reach a higher sugar yield, usually means a higher degree of sugar degradation. A compromise is needed between sugar yield and the level of sugar degradation. What is more important depends on the applications and value of the different (by-)product streams.

Generally speaking, less sugar degradation and furfural formation is better and therefore the advantage of organic acids versus sulfuric acid is twofold: less sugar degradation and an organic by-product stream.

In this paper, the kinetics of the degradation of arabinose are studied in the presence of sulfuric, maleic, and fumaric acid, and of water alone. Experimental conditions such as temperature, reaction times, and arabinose concentration are similar to those found in the pretreatment of lignocellulose biomass like corn stover and wheat straw. To link to practical pretreatment, as well as to show relevance of arabinose and the chosen experiment conditions, conversion of arabinan to monomeric arabinose is determined using wheat straw as lignocellulosic feedstock in lab scale pretreatment.

2. Materials and methods

All chemicals, except where noted below, were obtained from Sigma–Aldrich (St. Louis, MO).

2.1. Experimental set-up of arabinose degradation

For assessing arabinose degradation in the presence of different acid catalysts, arabinose (Sigma A3131) was dissolved in de-ionized

water or in 50 mM aqueous acid solutions to generate an arabinose concentration of 5 g/L (33 mM). The acids used were maleic (M-0375), fumaric (F-19353) and sulfuric acid (Mallinckrodt 2468), and all used chemicals were of research grade. Degradation at temperatures of 150 and 170 °C was examined with reaction times ranging from 10 to 60 min. For each reaction temperature, triplicate experiments were conducted for each of the de-ionized water/acid conditions.

2.2. Arabinose degradation kinetics measurement

Due to the increased pressure at elevated temperatures (a vapor saturation pressure of water of ~5 and 8 bars at 150 and 170 °C, respectively) [31] and the mechanical stress of rapid temperature changes on the reactors, all kinetics experiments were carried out in modified miniature glass reactor tubes. The reactor tubes were constructed using 12 mm × 32 mm crimp top HPLC vials (Alltech, Nicholasville, KY) with the seal reinforced by the addition of a piece of 0.075 mm (0.003 in.) brass sheet fitted between the original seal and the crimp cap. Each reactor has a 2.0 mL total volume, with a 1.5 mL working volume (at room temperature) to allow head space for liquid thermal expansion. Temperature control was achieved utilizing a Techne SBS-4 fluidized sand bath (Cole–Parmer, Vernon Hills, IL). The heat-up time was considered to be insignificant due to the very small size of the reactor vials (1.5 mL content). After the selected reaction time, the reactor vials were cooled by quenching in 20 °C water. After the reactors were cooled down, the content was filtered through a 0.20- μ m nylon filter (Fisherbrand), diluted to an appropriate concentration and further analyzed by the HPLC system described below.

2.3. HPLC analysis in degradation experiments

Samples were analyzed for arabinose, organic acids, and furfural concentrations by HPLC. Sample analysis utilized a Bio-Rad HPX-87H (300 mm × 7.8 mm) organic acid column (Bio-Rad Laboratories Inc., Hercules, CA) in a HPLC system consisting of a Rainin pressure module and Rainin solvent delivery system (Rainin Instrument, Oakland, CA), Waters 717 plus autosampler, Waters 2414 refractive index detector, Waters 2487 dual λ absorbance detector set at 280 nm (Waters Corp., Milford, MA), and a personal computer with Empower software (Waters Corp., Milford, MA, USA) for data processing and storage. The mobile phase was 5 mM sulfuric acid in distilled, de-ionized water filtered through 0.2 μ m filters. The operating conditions for the HPLC column were 70 °C with a mobile phase flow rate of 0.6 mL/min. Complete sample elution was accomplished within 48 min per injection. Arabinose and organic acids were measured by refractive index and furfural by UV absorption. Standard curves were obtained by dissolving pure compounds (>99% purity) in the mobile phase. Fractional dilutions of the standard solution were prepared to give calibration curves against peak area for arabinose (0.125–4.000 g/L), organic acids (0.125–4.000 g/L), and furfural (Fluka 48070) (0.0116–0.148 g/L). When the linear regressions for the calibration curves were computed, R^2 values were >0.9999 in all cases.

2.4. Preparation and analysis of wheat straw

Wheat straw (harvest September 2006, Delfzijl, The Netherlands) was milled twice; first in a Pallmann mill (4 mm × 30 mm sieve) and then in a Retsch mill (1 mm sieve). Milled straw was kept in a sealed plastic barrel at room temperature until used. Chemical composition was analyzed as described by TAPPI methods [32–37], with minor modifications. Samples were extracted with

ethanol:toluene 2:1, 96% (v/v) ethanol and hot water (1 h) at boiling temperature. The extracted samples were dried at 60 °C for 16 h. Monomeric sugar and lignin content of the ethanol-extracted material was determined after a two-step hydrolysis with sulfuric acid (12 M for 1 h at 30 °C; 1 M for 3 h at 100 °C). The acid soluble lignin in the hydrolyzate was determined by spectrophotometric determination at 205 nm. Monomeric sugars were measured by HPAEC-PAD (High Performance Anion Exchange Chromatography with Pulsed Amperometric Detection). A Dionex system with CarboPak PA1 column with pre-column was used at 30 °C, with de-ionized water as mobile phase (1 mL/min) and fucose as internal standard. For comparison purposes, the Dionex HPLC method was also used for determination of monomeric arabinose in the liquid phase of both pretreated and enzymatically hydrolyzed wheat straw. Dry matter content was 91.8% (w/w) (24 h at 105 °C). On dry matter base, the straw composition was: 36.3% cellulose, 23.2% hemicellulose, 25.5% lignin, 3.3% protein, 7.8% extractives, and 6.7% ash (w/w). The arabinan content was 2.1% (w/w).

2.5. Wheat straw pretreatment

Milled wheat straw (8.0 g) was mixed in poly-ethylene containers with 65.5 mL of acid solution (50 mM) or with de-ionized water, resulting in 10% (w/w) straw solid loading. Acids used were maleic acid (M-153), fumaric acid (F-19353) and sulfuric acid (Fluka 84721). The straw/acid mixture was soaked for 20–24 h at room temperature and then transferred to 316 L stainless steel reactors (inner height × diameter: 90.0 mm × 40.0 mm; 5.0 mm wall), fitted with thermocouples. Four reactors at a time were heated in a Haake B bath with a Haake N3 temperature controller (Thermo Fisher Scientific, Waltham, MA), filled with silicon oil (DC 200 fluid, 100 cSt, Dow Corning, Midland, MI). Sample core temperature was recorded (Picotech data collector and software; Picotech, UK). Holding time was 30 min, starting from when desired core temperature was reached. Heating bath oil was preheated to 100 °C; the temperature difference between the oil and the inside of the reactor did not exceed 10 °C during heat up, and not more than 1 °C during the holding time. After the reaction time, the reactors were cooled by quenching in ice water. Duplicate experiments were conducted.

2.6. Enzymatic hydrolysis of pretreated wheat straw

After pretreatment, reactor contents were transferred to 250 mL baffled shake flasks. De-ionized water was added to dilute to 5% (w/w), based on straw dry weight, taking into account water added during pH adjustment to 5.0 with 0.1 and 1 M NaOH solution, and water added with addition of 0.4 mL per g dry matter straw of GC220 cellulase enzyme mixture (Genencor, Rochester, NY) at the start of the enzymatic hydrolysis. Flasks were left overnight for the pH to equilibrate. After pH fine-tuning and enzyme addition, flasks were closed with airtight plugs and placed in an Innova 44 incubator shaker (50 °C, 150 rpm, 2 in. stroke; NBSC, Edison, NJ). Samples of 1.5 mL were taken at $t=0$ and 72 h; after 5 min enzyme inactivation at 90 °C, samples were stored at –20 °C until arabinose analysis.

The arabinose yield was calculated as follows:

$$\text{Arabinose yield}(\%) = \frac{\text{AH}}{\text{AS}} \times 100 \quad (1)$$

where AS is the arabinan content (%) of the dry straw (g arabinose/g dry matter straw), and AH is the arabinose content (%) of the hydrolyzate supernatant (g arabinose/g dry matter straw).

Table 1

Arabinose yield (%) from acid pretreated wheat straw.

	After pretreatment	After enzymatic hydrolysis
De-ionized water	15 (±0.3)	33 (±0.6)
50 mM fumaric acid	56 (±0.8)	62 (±0.5)
50 mM maleic acid	71 (±0.6)	72 (±1.1)
50 mM sulfuric acid	80 (±0.5)	79 (±1.3)

Acid pretreatment: 30 mins at 150 °C; 50 mM acid; 10% (w/w) dry straw solids loading. Between brackets: deviation from average.

3. Results and discussion

3.1. Arabinose from wheat straw

Wheat straw was pretreated at 150 °C, in the presence of 50 mM of sulfuric, maleic, and fumaric acid, or water alone. The formation of arabinose monomers was measured after pretreatment and subsequent enzymatic hydrolysis, and expressed as percentage of arabinose yield (see Table 1). Maximal yield means that all of the 2.1% (w/w) arabinosyl groups in the straw were hydrolyzed.

The stronger the acid in the pretreatment, the more arabinosyl side chains were converted to arabinose. Up to 80% arabinose yield was reached, after pretreatment with sulfuric acid; while using the organic acids results in a little less free arabinose. This shows that most of the arabinosyl side chains in the hemicellulose fraction of wheat straw are released as fermentable monomers. It also confirms the significance of arabinose contribution for improving the overall yield in lignocellulosic ethanol production. The 80% arabinose yield under the tested conditions corresponds very well with literature values, for example on wheat bran [14].

The enzymatic hydrolysis did not increase the arabinose yield much, or not at all in the case of maleic and sulfuric acid pretreatment. Acid strength during pretreatment had more effect than subsequent enzymatic treatment. The fact that the 70% yield during maleic acid pretreatment was not raised by the enzymatic treatment, while 80% arabinose was released during the sulfuric acid pretreatment, suggests the possibility that some arabinosyl side chains were still remaining after the maleic acid pretreatment, and were not released during the enzymatic treatment. This would mean that it may not have been extensive arabinose degradation that was limiting the arabinose yield during the maleic acid pretreatment, but that the subsequent enzymatic hydrolysis to arabinose itself was limited.

3.2. Arabinose degradation in solution

While in this study most of the arabinosyl groups are converted to arabinose during the acid pretreatment of wheat straw, degradation of the resulting sugar lowers the potential ethanol yield while also generating fermentation inhibiting furfural. Therefore, a closer examination of arabinose degradation to furfural in the presence of these acids was conducted. Arabinose degradation was examined in the presence of the same acids used for pretreatment: fumaric, maleic and sulfuric acid. As a control arabinose degradation was also measured in the presence of de-ionized water only. Samples were heated for 10, 20, 30 and 60 min at 150 and 170 °C. The degradation rate of arabinose is modeled as first-order with respect to arabinose and as zero-order with respect to the degradation product furfural, which leads to:

$$-\frac{dC_A}{dt} = k_d C_A \quad (1)$$

where C_A = arabinose concentration (g/L) and k_d = first-order degradation rate constant (min^{-1}). All experiments were performed with an initial arabinose concentration of 5 g/L (33 mM). The measured

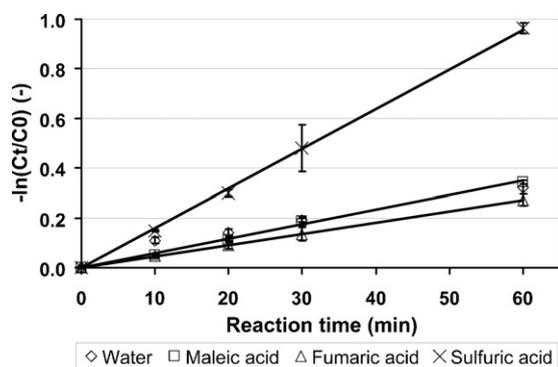


Fig. 1. Arabinose degradation at 170 °C, all acids at 50 mM. Error bars: 95% confidence interval.

residual arabinose concentration in the solution is expressed as a ratio over original concentration. The $-\ln(C_t/C_0)$ versus time plot is shown in Fig. 1 for a reaction temperature of 170 °C, with the slope of the graph representing the degradation rate constant k_d (min^{-1}). The calculated degradation rate constants for all experimental conditions can be found in Table 2. A Student's *t*-test was performed ($P < 0.01$) to determine if differences between degradation rate constants were statistically significant [38].

At 150 °C, sulfuric acid has a larger degradation rate constant than the two organic acids (Table 2). The difference between the rate constants of fumaric and maleic acid was not statistically significant ($P < 0.01$). However, the presence of the organic acids resulted in a significantly smaller reaction rate constant than when water alone was present. At 170 °C, all degradation rate constants were larger than at 150 °C (Fig. 1 and Table 2). Sulfuric acid (50 mM) showed a larger rate constant and therefore a higher degradation rate than maleic acid. Maleic acid resulted in a larger rate constant than fumaric acid. When only water was present, the resulting rate constant was equal to that of maleic acid, significantly larger than that of fumaric acid and significantly smaller than that of sulfuric acid.

Comparing degradation rate constants from earlier studies on glucose and xylose (Table 2), at 170 °C in the presence of both 50 mM sulfuric and maleic acid, the degradation rate constants for arabinose were smaller than those found for xylose by Lu and Mosier [25]. The same is the case at 150 °C. The results of the present study indicate that arabinose is more stable than xylose under the tested conditions (acid catalysts and temperature). Concerning glucose, the stability of this sugar seems to lie in between those of arabinose and xylose, when maleic acid is present at 170 °C. At 170 °C in the presence of sulfuric acid, glucose appears to degrade more readily than xylose or arabinose. However, the glucose results from Mosier et al. [39] were obtained using stainless steel reactors while the xylose and arabinose results were obtained using glass reactors. Differences in degradation rate of xylose at similar pH in the presence of different inorganic catalysts have also been previously noted, with Fe^{3+} being a strong catalyst for the degradation of xylose [40].

Table 2

Calculated degradation rate constants k ($\times 10^{-3} \text{ min}^{-1}$) of arabinose degradation, compared with previously published data for glucose and xylose degradation rate constants (50 mM acid).

	Arabinose		Xylose		Glucose	
	150 °C	170 °C	150 °C	170 °C	150 °C	170 °C
De-ionized water	1.49 (± 0.12)	5.81 (± 0.53)	n.a.	n.a.	n.a.	n.a.
50 mM fumaric acid	0.61 (± 0.23)	4.52 (± 0.11)	n.a.	n.a.	n.a.	n.a.
50 mM maleic acid	0.92 (± 0.27)	5.81 (± 0.13)	1.83	11.04	1.86	8.48
50 mM sulfuric acid	2.56 (± 0.38)	15.9 (± 0.32)	5.02	19.08	8.48	38.7

Between brackets: limits of 95% confidence interval. Xylose data from Lu and Mosier [25], glucose data from Mosier et al. [39], n.a.: data not available.

Table 3

pK_a values of acids and measured pH of reaction mixtures at room temperature [31].

	pK_a	pH at 50 mM acid, 5 g/L arabinose in water
Fumaric acid	3.02/4.38	2.20
Maleic acid	1.92/6.23	1.86
Sulfuric acid	-3/1.99	1.43

Thus in comparing results from different studies, differences in the reactor construction and the possible influence of metal catalysts (especially steel) may bias the data.

In the present study, at 150 °C the degradation rate constants from maleic and fumaric acid were not significantly different, but they were at 170 °C (maleic acid being higher). One explanation for this is the difference in pH between fumaric and maleic acid solutions is greater at 170 °C than at 150 °C [41]. These results may also suggest that the activation energy for arabinose degradation in the presence of maleic acid is higher than the activation energy in the presence of fumaric acid [5,25,42,43].

At an acid concentration of 50 mM, the arabinose degradation rate at 170 °C differs depending on the pK_a of the acid used (Table 3). The stronger the acid, the larger the resulting degradation rate constant of arabinose. However, at 170 °C, the degradation rate constant of arabinose in the presence of 50 mM maleic acid is equal to that when only water is present. This means that 50 mM maleic acid shows no extra catalytic power to that of water and fumaric acid acts to stabilize arabinose. Similar behavior has been noted for glucose degradation by Mosier et al. [39]. There it was found that even at 100 and 200 mM of maleic acid, the degradation rate was very close to that of water alone. The result that a presumed acid-catalyzed degradation is catalyzed by 50 mM of sulfuric (strong) acid and not by 50 mM of a weak acid suggests that the degradation is not a standard specific acid (H^+) catalyzed reaction. Mosier et al. [39] found that a minimal amount of catalyst donated H^+ (not from water) was needed to increase degradation rates above the baseline (water alone). When sulfuric acid concentrations are below 25 mM the rate of degradation of glucose approached the rate caused by water alone. Possibly, a similar minimal amount is needed to catalyze arabinose degradation.

When 50 mM fumaric acid is present, the arabinose degradation rate constant at 170 °C is smaller than when only water is present. Here, the presence of the acid seems to diminish the catalytic behavior of water. At 150 °C, both organic acids seem to diminish the catalytic behavior of water. An explanation for this may have to do with an influence of the anion. The anion may influence the degradation by inhibiting the protonation of the hydroxyl group (Fig. 2), which is the rate-limiting step in sugar degradation [24].

It can be argued that it is the degradation in the presence of water alone that does not follow the general trend, and that all the tested acids are showing results that can be expected from a specific acid catalysis; larger rate constants as the pH decreases. The catalytic action of water alone cannot be explained by acid catalysis and the increase in K_w at higher temperatures. When the temperature is raised from 25 to 150 °C, the pK_w decreases from 14 to

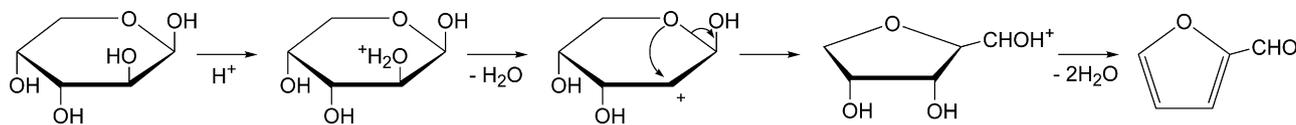


Fig. 2. Degradation mechanism of arabinose to furfural (based on Nimlos et al. [21]).

11.6 and the H^+ concentration increases by a factor 230 to around 1.5×10^{-3} mM [31]. However, this is still far from the H^+ concentration present in the reaction mixture with 50 mM fumaric acid at 25°, namely 6.3 mM. The K_a of carboxylic acids decreases as temperature rises from 25 to 150 °C or higher [41], but only by a factor of 3–4; not to the same extent as the increase of the K_w of water. Another mechanism for sugar degradation is a possibility. Nucleophilic attack of water and/or acid anion molecules on the sugar or the degradation intermediates (Fig. 2) may account for these observations, but this remains undetermined. Further studies are needed to clarify the mechanism(s) of arabinose degradation in the presence of acids.

4. Conclusions

This study suggests using fumaric or maleic acid for biomass pretreatment instead of sulfuric acid has advantages. Mainly, because of the absence of catalytic action of the organic acids in arabinose degradation, less fermentation inhibiting furfural could improve the total efficiency of the ethanol production process. Indications are presented that the organic acids may even diminish the arabinose degradation, compared to water alone.

In addition, it is shown that during the maleic and fumaric acid pretreatment of wheat straw, most of the arabinosyl side chains in the hemicellulose fraction are released as fermentable arabinose to improve yields in ethanol production. The arabinose release is somewhat less than in the case of sulfuric acid, but the by-product stream is kept free of sulfur. Another interesting point is that arabinose degrades less readily than xylose and glucose. This difference may contribute to a better understanding of the mechanism of sugar degradation into fermentation inhibiting products during the pretreatment of cellulosic plant biomass.

All in all, it is clear from this study that careful selection of acid properties plays an important role in creating the most efficient acid pretreatment process.

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