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

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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.



## 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 of 15% xylan and 3% arabinan, wheat straw contains 19% xylan and 2% arabinan, whereas wheat bran contains 19% xylan and 15% arabinan

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[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-μm 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 μm 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 be 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