

Reintroduced Solids Increase Inhibitor Levels in a Pretreated Corn Stover Hydrolysate

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Abstract

Following detoxification of the liquid hydrolysate produced in a corn stover pretreatment process, inhibitor levels are seen to increase with the re-addition of solids for the ensuing hydrolysis and fermentation processes. The solids that were separated from the slurry before detoxification of the liquor contain approx 60% (w/w) moisture, and contamination occurs owing to the diffusion of inhibitors from the moisture entrained in the porous structure of the corn stover solids into the bulk fluid. This evidence suggests the need for additional separation and detoxification steps to purge residual inhibitors entrained in the moisture in the solids. An overliming process to remove furans from the hydrolysate failed to reduce total organic acids concentration, so acids were removed by treatment with an activated carbon powder. Smaller carbon doses proved more efficient in removing organic acids in terms of grams of acid removed per gram of carbon powder. Sugar adsorption by the activated carbon powder was minimal.

Index Entries: Activated carbon; detoxification; organic acids; overliming; pretreated corn stover hydrolyzate.

Introduction

Cellulosic crops and other sources of biomass harvested for the conversion to fuels and chemicals do not contain significant quantities of readily fermentable simple sugars. The development of economically viable processes for the extraction of simple sugars from biomass is ongoing and there is room for improvement in all phases of the conversion process, especially the hydrolysis of hemicellulose and cellulose and the subsequent removal of undesirable byproducts. Acid treatments that hydrolyze hemicellulose and prepare the cellulose for subsequent enzymatic hydrolysis have been studied for some time. Dilute acid hydrolysis is

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currently the most common form of biomass pretreatment. Often in a dilute acid hydrolysis process, the biomass is first ground into small pellets and then soaked in a dilute acid, usually sulfuric acid (1). The pretreatment of most biomass systems causes degradation of sugars and lignin resulting in the formation of byproducts such as furfural, hydroxy methyl furfural, phenolic compounds, acetic acid, and other organic acids. Although these chemicals are not always produced in great quantities, they can have a toxic effect on the fermentative ability of ethanologenic organisms, especially bacteria (2). Concentrations of acetic acid found in pretreated softwoods may reach 10 g/L, completely inhibiting the fermentative ability of *Zymomonas mobilis* (3).

Several detoxification methods have been investigated to overcome the toxic effects of these byproducts (4–9). Overliming is a common and effective process in which the pH of hot hydrolysate is adjusted with lime resulting in the formation of insoluble salts (4–6). Although common and effective, overliming does not affect acetic acid levels (5), which are known to inhibit ethanol production above 2 g/L (10). Detoxification by activated carbon adsorption has proven to effectively remove acetic acid from a synthetic hydrolysate solution formulated to mimic the composition of an actual hydrolysate (11) and an actual corn stover hydrolysate (12). Carbon has been used as an adsorbent for hundreds of years because it was discovered that it could be used to purify drinking water and remove color impurities and is still commonly used today in wastewater, drinking water, refinery waste, and chemical clarification applications (13). Activated carbons are regenerated easily with steam, and the stripped components may be recovered and marketed. A number of studies have qualitatively reported using activated carbons on acid-hydrolyzed wood and sugar cane substrates before fermentation with varying degrees of success (14–17).

Overliming and carbon adsorption procedures are performed in liquid hydrolysates from which biomass solids are necessarily first separated to prevent interference during the detoxification procedure. The cellulose containing solids are added back to the conditioned liquor for the enzymatic hydrolysis of cellulose and fermentation. In this study, the effect of reintroducing corn stover solids on inhibitor levels in conditioned hydrolysates is investigated. Conditioning is performed by overliming and carbon adsorption methods. Furans and organic acids are measured before detoxification, following detoxification, and following the reintroduction of solids. The effect of carbon loading on adsorption efficiency is also examined.

Materials and Methods

A corn stover slurry pretreated with dilute sulfuric acid (190°C, 1.6% acid, 30% solids) was provided by the National Renewable Energy Laboratory. The slurry viscosity is on the order of 25,000 cP or higher at

room temperature and a shear rate of 1/s, and because of the impracticality of dispersing carbon or $\text{Ca}(\text{OH})_2$ solids in this viscous material, liquid hydrolysate is necessarily first separated from the slurry. The solids are later returned to the detoxified liquid. Vacuum filtration is employed for the separation process. After maximum liquid separation from the vacuum filtration, the solids were found to contain 60% moisture. Overliming was performed by raising the liquor temperature to 50°C, manually adding $\text{Ca}(\text{OH})_2$ until the pH reached a value of 10.0, and maintaining these conditions for 30 min. Total furans, before and after overliming, were measured using the UV spectra method described by Martinez et al. (18) in a Genesys 20 spectrophotometer from ThermoSpectronic (Waltham, MA). The UV spectra method provides a reasonable estimation of relative concentrations based on the difference in absorbance readings between 284 and 320 nm, so instead of quantifying absolute furans concentrations, results are reported in terms of ΔA ($A_{284} - A_{320}$).

Calgon BL activated carbon powder provided by Calgon Carbon Corporation (Pittsburgh, PA) was used for organic acid adsorption testing. Tests with carbon concentrations equal or less than 80 g/L were run in an Innova Model 4230 benchtop refrigerated incubator/shaker from New Brunswick Scientific (Edison, NJ). The incubator contains an Erlenmeyer flask platform capable of holding 25 × 250 mL flasks. Flasks were loaded with 100 mL of liquid and carbon powder concentrations of 20, 40, or 80 g/L. The shaker was operated at 250 rpm and 30°C. Flasks were covered with parafilm to prevent evaporation. Tests at higher carbon concentrations were mixed with a Lightnin Mixer fitted with a R100 impeller to prevent carbon aggregation. After 30 min of carbon treatment in the hydrolysate, carbon was separated from the liquid by centrifugation followed by passing the remaining liquid through a 0.1 µm filter (diameter of carbon particles is 150 µm). Initial and equilibrium total organic acids concentrations were measured by titration with NaOH. To determine the extent of sugar adsorption by the carbon, glucose, and xylose compositions were measured using an Alltech HPLC equipped with a Biorad Aminex HPX-87H column and ELSD detector.

Results and Discussion

Previously, attempts at fermenting a broth containing detoxified hydrolysate with cellulosic rich corn stover solids returned to the liquor resulted in lower ethanol production than expected (19) based on fermentations in synthetic liquid broths containing varying levels of inhibitors (10). The moisture (60%) in the returning solids should contain inhibitors similar to the preconditioned bulk fluid, so it was postulated that diffusion from the moisture in the pores of the solid back into the conditioned bulk fluid would cause a rise in bulk fluid inhibitor levels.

Total furans were measured before an overliming process, following an overliming process, and following the addition of varying levels of corn

Table 1
Effect of Reintroduced Solids on Total Furans
Concentration After Overliming

Stage	Total furans in hydrolysate ($A_{284}-A_{320}$)
Initial	0.393
Overlimed	0.001
10% Solids added	0.139
15% Solids added	0.164
17% Solids added	0.175
20% Solids added	0.205

stover solids after overliming appear in Table 1. The solids added back to the hydrolysate were allowed to equilibrate for 2 h. Furans (after dilute acid pretreatment) were reduced from an initial ΔA of 0.393 to 0.001 following the overliming process. After 100 g/L corn stover solids were reintroduced into the hydrolysate, ΔA increased to 0.139. After reintroduction of 150 g/L, 170 g/L, and 200 g/L solids, ΔA measured 0.164, 0.175, and 0.205, respectively. Although the actual furans concentrations are not quantified, the increase owing to the reintroduction of solids is clearly illustrated, a 17.6% increase in reintroduction of solids (170 to 200 g/L) results in a 17.1% increase (0.175 to 0.205) in relative furans concentration.

Total organic acid levels increased as well when filtered corn stover solids were returned to the conditioned hydrolysate. Results presented in Fig. 1 show the effect of reintroduced solids concentration on the new equilibrium concentration of total organic acids in the bulk liquid. Initial acid concentration (approx 18 g/L) was reduced to 6 g/L through a series of activated carbon treatments using 80 g/L carbon per step. After the carbon was separated from the hydrolysate, corn stover solids were reintroduced and allowed to equilibrate for 2 h. Tests were performed using 100, 150, and 200 g/L corn stover solids. The solids were filtered from the hydrolysate to allow titration for organic acids concentration.

The vertical lines after the fourth treatment step represent the increase in organic acids following the reintroduction of solids. The reintroduction of 100 g/L solids increased the organic acids concentration from 6 to 8.3 g/L. The reintroduction of 150 g/L solids increased the organic acids concentration from 6 to 10.3 g/L. The reintroduction of 200 g/L solids increased the organic acids concentration from 6 to 11.9 g/L. The correlation between organic acids increase and mass of solids added is approximately linear. A 50% increase from 10 to 15% solids resulted in a 53% increase in organic acids (4.3 g/L vs 2.3 g/L), and a 33% increase from 15 to 20% solids resulted in a 37% increase in organic acids (5.9 g/L vs 4.3 g/L). The concentration gradient between the liquid in the pores of the reintroduced solids and the bulk liquid will determine the new equilibrium concentration of a component in the bulk liquid, with material diffusing from

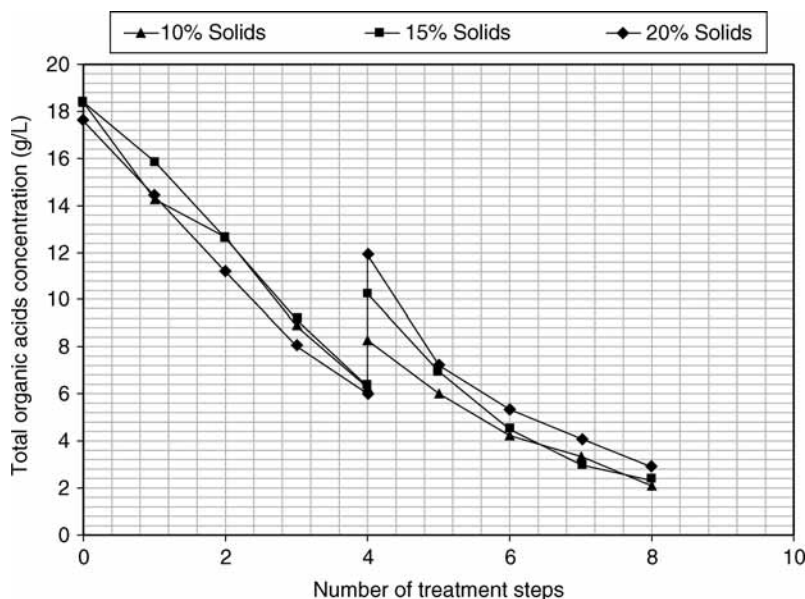


Fig. 1. Effect of reintroduced solids on bulk fluid total organic acids concentration.

Table 2
Effect of Initial Organic Acids Concentration on Final Organic Acids Concentration After the Reintroduction of 20% Solids

Initial total organic acids (g/L)	Final total organic acids (g/L)	Change (g/L)
11.6	13.1	1.5
10.9	13.2	2.3
6	11.9	5.9

the pores of the solids in which untreated liquid resides into the bulk liquid in which the inhibitor concentrations have been reduced. Therefore, it would be expected that inhibitor levels will increase more when solids are added to hydrolysate with lower initial inhibitor levels. In this context, "initial" refers to the time immediately before the reintroduction of solids.

Table 2 shows the change in organic acids levels after the reintroduction of 200 g/L solids into hydrolysate with varying initial organic acids concentrations. Initial organic acids concentrations were established by altering the number of carbon treatment repetitions; fewer repetitions removed less acid resulting in higher initial acid concentrations. Data in the table shows the expected trend: for initial organic acids concentrations of 11.6, 10.9, and 6.0 g/L, final equilibrium concentrations increased by 1.5, 2.3, and 5.9 g/L. Inhibitors are removed from hydrolysate for the benefit of the downstream fermentation and the increase in inhibitors following the reintroduction of solids indicates the need for additional detoxification steps, or a means of

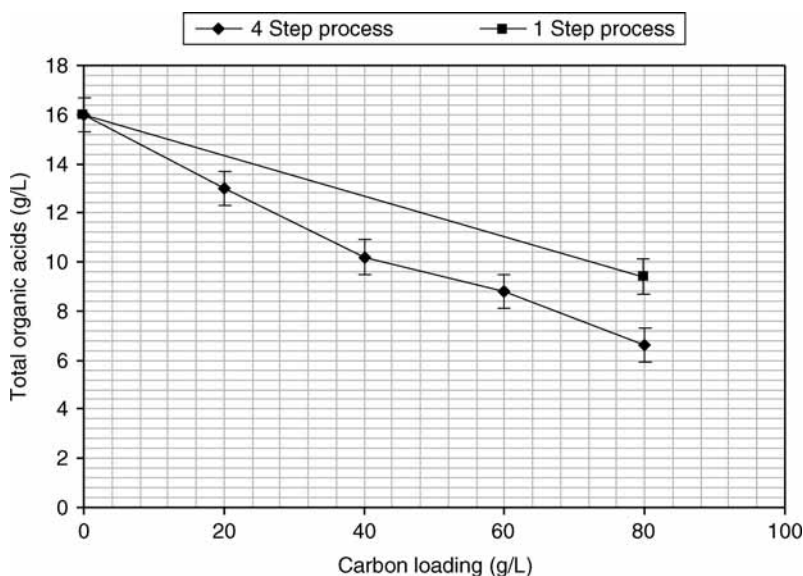


Fig. 2. Carbon treatment efficiency comparison (80 g/L total carbon).

purging inhibitors directly from the solids through a washing process. If additional detoxification steps are favored, the process of detoxifying and reintroducing solids may need to be repeated until inhibitor levels stabilize.

Previously reported adsorption isotherm data (12) suggests that lower activated carbon concentration may be more efficient at removing organic acids in terms of acid removed per gram of carbon. This was verified by performing a single step detoxification with a given carbon loading and comparing the final organic acids concentration to the final concentration in a test in which an equivalent amount of total carbon was divided into four batches and four consecutive detoxifications were performed. The hydrolysate used in these two tests originated from the same batch of pretreated corn stover so the initial compositions were equivalent.

Results in Fig. 2 indicate organic acids concentration was lowered from 16 to 9.4 g/L using 80 g/L carbon in a single step and from 16 to 6.6 g/L using 20 g/L carbon in four consecutive steps, an improvement of 42% adsorption. The improvement was even greater in tests with lower total carbon loading. When 10 g/L carbon in four consecutive steps is in comparison with a single 40 g/L step, organic acids removal improved by 55% (7 g/L removed vs 4.5 g/L), and when 5 g/L carbon in four consecutive steps is in comparison with a single 20 g/L step, organic acids removal improved by 117% (3.5 g/L removed vs 7.6 g/L). The final organic acids levels are above known critical limits for *Z. mobilis* growth, but the data is intended to illustrate the differences in efficiency for varying carbon concentration levels.

With fewer carbon particles present in the liquid at lower carbon loadings, a higher concentration gradient exists between organic acids in

Table 3
Carbon Treatment Efficiency Comparison—High Shear Environment

100 g/L carbon/stage × 2 stages		50 g/L carbon/stage × 4 stages		25 g/L carbon/stage × 8 stages	
Stage	Acid (g/L)	Stage	Acid (g/L)	Stage	Acid (g/L)
Initial	16.2	Initial	16.2	Initial	16.2
1	9.8	1	13.3	1	14.7
2	4.6	2	9.7	2	12.4
		3	6.1	3	11.1
		4	3.9	4	9.8
		5	7.9		
		6	6.4		
		7	4.8		
		8	3.6		

the bulk fluid and each carbon particle, driving more acid into each particle. This may be evidence that the rate limiting step of adsorption of organic acids by the carbon particles is diffusion across the bulk liquid-particle surface interface rather than diffusion within the particle pores.

If carbon particles aggregated while mixing in the shaker flasks during the adsorption treatment process, which would be more likely at higher carbon loadings, the total surface area of carbon will be reduced, which would reduce the efficiency at higher carbon loadings. To address this, a test was performed in which carbon was mixed in the hydrolysate with a high-shear R100 impeller at 250 rpm instead of mixed in shaker flasks to prevent aggregation. Results in Table 3 for this high-shear system are consistent with shaker flask tests; organic acids adsorption occurs with higher efficiency when equivalent total carbon loading is distributed among more treatment steps, ruling out aggregation of carbon particles as a cause for reduced efficiency with higher carbon concentration.

Because the removal of organic acids by the carbon treatment process will not be considered useful if significant amounts of sugars are removed, xylose and glucose concentrations were measured before and after treatment to determine the extent of their adsorption. Xylose is present in the acid-treated hydrolysate at 30.5 g/L and glucose is present at 3.7 g/L. Following hydrolysate conditioning by carbon treatment, xylose, and glucose concentrations were remeasured (Table 4). Depending on carbon loading, xylose concentration was reduced by 1.3 to 3.9 g/L and glucose concentration was reduced by 0.3 to 0.5 g/L. The loss of xylose and glucose from hydrolysate is comparable to losses reported owing to overliming (6). The carbon's affinity for organic acids and lack of affinity for xylose and glucose may be attributed to the size of the molecules. Although the size of the pore opening is unknown, the molecular weights of xylose, 150 g/mol, and glucose, 180 g/mol, are

Table 4
Adsorption of Sugars During Carbon Treatment

Carbon load (g/L)	Xylose (g/L)	Glucose (g/L)
Initial	29.7	4.1
80	25.8	3.6
40	28.4	3.8
20	26.9	3.7

two and a half and three times the size of the molecular weight of acetic acid, 60 g/mol.

Conclusions

Following the reintroduction of corn stover solids into detoxified hydrolysate, total furans and organic acids levels increased significantly enough to require additional treatment before fermentation. The amount of increase depended on the amount of solids returned and the existing concentration of the inhibitor. Carbon efficiency for removing organic acids was higher for lower carbon loadings. Adsorption of both xylose and glucose by the carbon was minimal.

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