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Detoxification of Actual Pretreated Corn Stover Hydrolysate Using Activated Carbon Powder

R. Eric Berson,*[,1] John S. Young,[1] Sarah N. Kamer,[1] and Thomas R. Hanley[2]

[1]*Department of Chemical Engineering, University of Louisville, Louisville, KY 40292, E-mail: eric.berson@louisville.edu; and*
[2]*Office of the Provost, Auburn University, Auburn, AL 36849*

Abstract

A technique for the removal of acetic acid from an actual pretreated corn stover hydrolysate was investigated. A powdered form of activated carbon previously shown to be effective in the removal of acetic acid from a synthetic hydrolysate was utilized. The method proved to be effective at lowering acetic acid levels while exhibiting minimal adsorption of the desired sugars from the hydrolysate, although at a lower efficiency in the actual hydrolysate than in the synthetic hydrolysate. Results are obtained for temperatures between 25 and 35°C and agitation rates between 150 and 350 rpm in shake flasks. Adsorption isotherm and kinetic rate data are presented. Temperature differences over this range did not have an effect on adsorption characteristics. Five stages of detoxification were necessary to lower acetic acid concentration to the maximum 2 g/L desired for fermentation.

Index Entries: Acetic acid; activated carbon; adsorption isotherms; detoxification; pretreated corn stover hydrolysate.

Introduction

Cellulosic crops, agricultural residue, and wood are all abundant sources of biomass for the extraction of sugars and their subsequent conversion into fuel ethanol via fermentation. Since these sources do not contain significant quantities of sugar in a simple fermentable state, technological concerns must be overcome in order to reduce these materials into fermentable sugars in a cost-effective manner. Technological concerns are the separation of lignin from the cellulose and hemicellulose, and then hydrolysis of the cellulose and hemicellulose into simple sugars.

Acid pretreatments prior to enzymatic hydrolysis have been studied for some time. Dilute-acid hydrolysis is the most commonly used form of

*Author to whom all correspondence and reprint requests should be addressed.

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 cau-ses degradation of sugars and lignin resulting in the formation of bypro-ducts such as furfural, hydroxymethyl furfural, and acetic acid. 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 including treatment with alkali, sulfites, and ion exchange *(4–7)*. Detoxification by activated carbon adsorption, which has received little attention for this application, may provide advantages such as ease of use and scale-up ability. Carbon has been used as an adsorbent for hundreds of years since it was discovered that it could be used to purify drinking water and remove color impurities *(8)*. Carbon adsorption is still commonly used today in wastewater, drinking water, refinery waste, and chemical clarification applications *(8)*. Activated carbons are much less costly than ion-exchange resins and 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 sugarcane substrates prior to fermentation with varying degrees of success *(9–12)*, and this method has proven to remove acetic acid from a synthetic hydrolysate solution effectively *(13)*. The synthetic solution did not contain sugars, so the possibility exists of unwanted simultaneous sugar adsorption.

In the present study, a technique for the removal of acetic acid from an actual dilute-acid-pretreated corn stover hydrolysate was investigated, and the results are presented quantitatively in the form of adsorption isotherms and kinetic rate data. A powdered form of activated carbon (Calgon BL) previously shown to be effective in the removal of acetic acid from a synthetic hydrolysate was utilized. The objective was to determine the effectiveness of the carbon powder in lowering acetic acid concentration below the 2 g/L level known to inhibit ethanol production while exhibiting minimal adsorption of sugars from the actual hydrolysate. Adsorption isotherms for acetic acid and glucose are presented as a means of comparing efficiencies. Calculated external film and pore diffusivity coefficients help explain isotherm characteristics. A corn stover slurry pretreated with dilute sulfuric acid was provided by the National Renewable Energy Laboratory (NREL).

Materials and Methods

The corn stover slurry provided by NREL was produced by a dilute sulfuric acid pretreatment (190°C, 1.6% acid, 30% solids) and has a thick,

sludgelike consistency. Because of the impracticality of dispersing carbon powder in a thick sludge, liquid hydrolysate is separated from the slurry. The solids can later be added back to the detoxified liquid for fermentation. Vacuum filtration is employed for the separation process. Although the remaining solids cannot be completely dried because this renders enzyme activity on the solids ineffective, a batch was dried and weighed to determine water content in the separated solids. This information is useful when recombining solids with detoxified liquid to a desired solids concentration. After maximum liquid separation from the vacuum filtration, the solids were found to contain 60% moisture.

Calgon BL activated carbon powder, provided by Calgon Carbon, was used for isotherm and kinetic rate testing. Tests were run in an Innova Model 4230 benchtop refrigerated incubator/shaker from New Brunswick Scientific. The incubator contains an Erlenmeyer flask platform capable of holding twenty-five 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 speeds between 150 and 350 rpm and temperatures between 25 and 35°C. The flasks were covered with Parafilm to prevent evaporation.

The hydrolysate/carbon powder mixture was allowed to mix for 2 h during adsorption isotherm testing. Then the carbon was separated from the liquid by centrifugation followed by passing the remaining liquid through a 0.1-μm filter (diameter of carbon particles: 150 μm). Initial and equilibrium acetic acid concentrations were measured by titration with NaOH. Initial and equilibrium glucose concentrations were measured in a YSI 2700 biochemistry analyzer.

The hydrolysate contained both sulfuric acid and acetic acid, and prior to carbon treatment the hydrolysate had a pH of 2.2. To ensure that these were the only significant components being titrated, a solution of sulfuric/acetic acid was mixed with the same relative concentrations as was measured in the hydrolysate and the corresponding titration curves were compared for consistency. Since the curves compared favorably with little variance, and the inflection points for both sulfuric acid and acetic acid occurred at the expected pH based on known pK_a values, it was concluded that no other significant components were being titrated.

For kinetic rate testing, the hydrolysate was sampled at 1, 2, 5, and 10 min. Preparation of media, separation of carbon from liquid, and sample measurements followed the same procedures as isotherm testing.

Model Development

The four steps of adsorption by a particle in solution are (1) mass transfer of solute through the bulk solution, (2) mass transfer from bulk solute to the particle surface, (3) intraparticle diffusion, and (4) adsorption on an interior site. A mathematical model is developed to describe the

adsorption rate data. Data for the model are collected during the kinetic testing phase. If a solution is well mixed, no concentration gradients exist within the bulk solution and this term may be ignored. It is assumed that adsorption on an interior site is rapid with respect to the remaining two steps *(14)*. The model, therefore, incorporates mass transfer of solute from the bulk liquid to the particle's surface (external film coefficient) and diffusivity within the particle (pore diffusivity coefficient). In a sufficiently mixed system (high agitation), external mass transfer rates become negligible compared to pore diffusivity rates, leading to an intraparticle diffusion controlled adsorption process.

Determination of external mass transfer coefficients begins with the following mass balance on a carbon particle *(14)*:

$$\frac{dC_b}{dt} = \frac{-3k_f m(C_b - C_s)}{R\rho_s(1-\varepsilon)} \tag{1}$$

For the limiting case of a linear isotherm at times close to t, $KC_S = [(C_{b0} - C_b)/m]$. A linear isotherm has been verified for this system for data collected in the first 10 min. Solving for C_S, substituting into Eq. 1, and using the initial condition $C_b = C_{b0}$ at $t = 0$ yields:

$$\left(\frac{C_b}{C_b}\right) = \frac{1}{1+mK} + \frac{mK}{1+mK}\exp\left[-\left(\frac{1+mK}{mK}\right)\left(\frac{3k_f mt}{R\rho_s(1-\varepsilon)}\right)\right] \tag{2}$$

A plot of ln $\{(C_b/C_{b0}) - [1/(1 + mK)]\}$ vs t is linear with a slope of $-\{[(1 + mK)/mK][3mK_f/R\rho_s(1 - \varepsilon)]\}$, from which k_f is calculated.

The determination of pore diffusivity begins with a shell balance on the system *(15)*. With the assumptions spherical particles, constant density, no reaction, no convection, constant diffusivity, and diffusion occurring only radially within the carbon particle, Fick's law of diffusion results:

$$\frac{dC}{dt} = D_e \frac{1}{r^2}\frac{\partial}{\partial r}\left(r^2 \frac{\partial C}{\partial r}\right) \tag{3}$$

The differential mass balance for this system is given in Eq. 4 for the initial condition: $C_b = C_b$ at $t = 0$ and $C = 0$ for $0 \le r \le R$.

$$V_f \frac{dC_b}{dt} = \frac{-3W}{R\rho_s} D_e \frac{\partial C(R,t)}{\partial r} \tag{4}$$

For an intraparticle diffusion controlled adsorption (high agitation), Eq. 3 is subject to the following conditions:
Initial condition:

$$t = 0 \quad C = 0 \quad \text{for } 0 \le r \le R$$

Boundary conditions:

$$t > 0 \quad \left.\frac{\partial C}{\partial r}\right|_{r=0} = 0 \quad \text{and} \quad C = C_b \text{ at } r = R$$

Solution of these equations is accomplished by a generalized Sturn-Liouville Integral transform resulting in Eq. 5 *(16)*:

$$\frac{C_b}{C_b} = \frac{B}{B+1} + 6B\sum_{n=1}^{\infty} \frac{\exp(-\xi_n^2 \tau)}{(9+9B+B^2\xi_n^2)} \tag{5}$$

in which the eigenvalues are determined from Eq. 6:

$$\tan \xi_n = \frac{3\xi_n}{3 + B\xi_n^2} \tag{6}$$

with

$$B = \frac{V_F}{WK} \tag{7}$$

The values t and τ are related through the dimensionless variable, $C_b/C_{b'}$ in which a plot of τ vs t yields a straight line with a slope equal to D_e/R^2, allowing the determination of pore diffusivity, D_e.

Results and Discussion

All of the adsorption isotherm data displayed in the results are fitted to the popular Freundlich isotherm equation, $q = KC^{1/n}$, for which Freundlich determined best fits isotherm data of organic systems *(17)*. Figure 1 shows basic adsorption information for the removal of acetic acid from an actual corn stover hydrolysate system. Data from the test with the highest agitation rate yield the highest intercept, indicating a higher capacity for the carbon to retain solute. The extra energy input to the system allows the solute to overcome resistances to diffusion within the carbon particle. The increase from 150 to 350 rpm resulted in approximately an extra 1 g/L of acetic acid being adsorbed by the carbon. The initial concentration of acetic acid in the hydrolysate was determined by titration to be 16.5 g/L. The final concentration in the 150-rpm system was 12 g/L and in the 350-rpm system was 11 g/L. This information can be used for future sizing and scale-up criteria by comparing extra energy requirements to the use of extra carbon to achieve an equivalent amount of adsorption.

Priddy and Hanley *(13)* recommended testing for differences in adsorption capacity at temperatures above 25°C based on findings with a synthetic hydrolysate. Figure 2 shows a general overlap of isotherms with

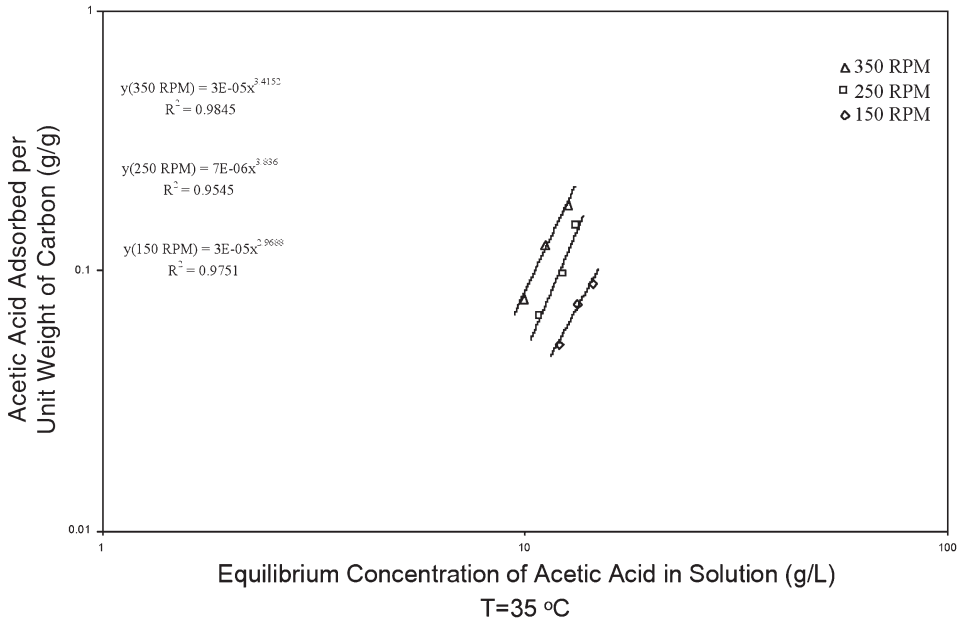

Fig. 1. Adsorption isotherms for removal of acetic acid from hydrolysate.

no clear indication of temperature having a significant effect on adsorption efficiency. Although the 30°C isotherm appears to indicate the highest capacity for adsorption of the three isotherms, there is no apparent reason or physical explanation to expect it to outperform the 35°C system. Although temperature differences would be expected to influence adsorption of a gas-phase solute, the range tested here appears to be too low to have a definitive effect on liquid acetic acid.

To help describe adsorption characteristics as related to temperature, external film and pore diffusivity coefficients were calculated for varying temperatures at constant agitation rate and carbon concentration. Kinetic rate data as seen in Fig. 3 are employed for the calculations as described in Eqs. 1–7.

Table 1 contains the coefficients for temperatures between 25 and 35°C at 250 rpm and 80 g/L of carbon. The difference between the diffusivity coefficients at 25 and 35°C is 9.4%, and the difference between the highest and lowest external film coefficients over this range is 1.1%. The small differences in both coefficient values support the assertion that temperature differences do not play a role in adsorption capacity in this system.

It is expected that diffusion in the particle pores would be rate limiting because the system should have sufficient mixing at 250 rpm to overcome the resistance of mass transfer from the bulk solution to the particle's surface. The film coefficient values exhibited very little change compared

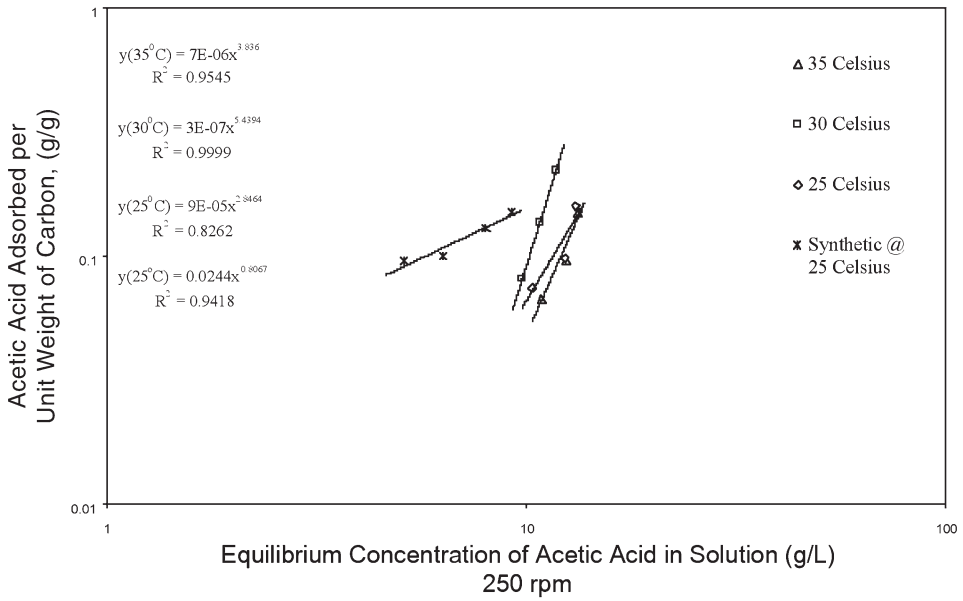

Fig. 2. Effect of temperature on adsorption isotherms and comparison with synthetic hydrolysate. (Synthetic data from ref. 18.)

with the diffusivity coefficient values, indicating that differences in adsorption are more likely linked to differences in internal diffusivity rather than external mass transfer.

The data in Fig. 3 also show that the higher the carbon concentration, the lower the adsorption efficiency. With 20 g/L of carbon, 3.6 g/L of acetic acid was removed from the hydrolysate after 5 min; with 40 g/L of carbon, 4.5 g/L of acetic acid was removed from the hydrolysate after 5 min; and with 80 g/L of carbon, 6.0 g/L of acetic acid was removed from the hydrolysate after 5 min. Therefore, a doubling of carbon use from 20 to 40 g/L yields only a 125 % increase in acetic acid removal, and a doubling of carbon use from 40 to 80 g/L similarly yields only a 133 % increase in acetic acid removal. A quadrupling of carbon yields only a 167 % increase in acetic acid removal.

A comparison between the efficiency of adsorption in the actual hydrolysate and the synthetic hydrolysate is shown in Fig. 2. The synthetic hydrolysate curve represents adsorption in a system initially containing 10 g/L of acetic acid in water. The actual hydrolysate initially contained 16.5 g/L of acetic acid in addition to other substances in unknown amounts formed during pretreatment. Both systems contained 80 g/L of carbon and operated at 250 rpm and 25°C. The position of the synthetic hydrolysate curve indicates a higher capacity of the carbon in the synthetic media to adsorb acetic acid than carbon in the actual hydrolysate. Additionally, the shallower isotherm slope for the synthetic media indicates

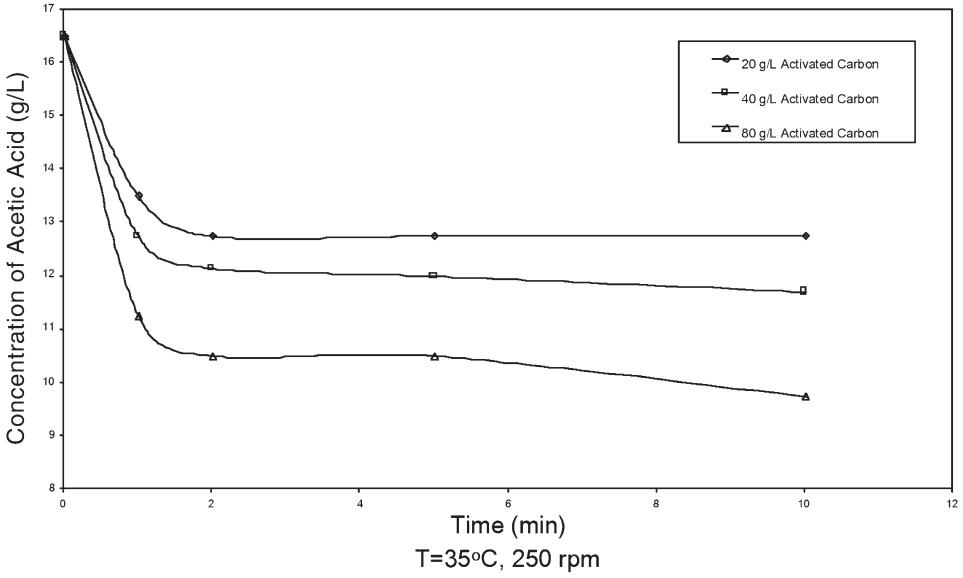

Fig. 3. Kinetic rate data.

Table 1
External Film and Pore Diffusivity Coefficients (250 rpm, 80 g/L)

Temperature (°C)	k_f (μm/s)	De (μm^2/s)
25	0.0180	0.431
35	0.0178	0.394

less dependence on solute concentration to perform the adsorption, and a smaller residual amount of solute will remain in the synthetic media than in the actual media at maximum adsorption conditions. The slopes do indicate a crossover point, at approximately double the current equilibrium solute concentration, where capacity of adsorption in the actual hydrolysate will be higher than in the synthetic hydrolysate. However, the desired final acetic acid level is at the lower end, so operating conditions will not occur at or greater than the crossover point.

Although it appears that the synthetic media exhibits more efficient adsorption, these results are to be expected. The actual hydrolysate contains organic substances other than acetic acid that the carbon will also adsorb. The competition for adsorption sites inside the carbon particles will limit the amount of acetic acid adsorbed. Furthermore, the presence of other substances in the bulk solution may cause a reduction in the driving force, inhibiting the movement of acetic acid toward the carbon particles.

A detoxification method for the removal of acetic acid and other inhibitors will not be considered useful if significant amounts of sugars are removed during the detoxification. Glucose is present in the pretreated

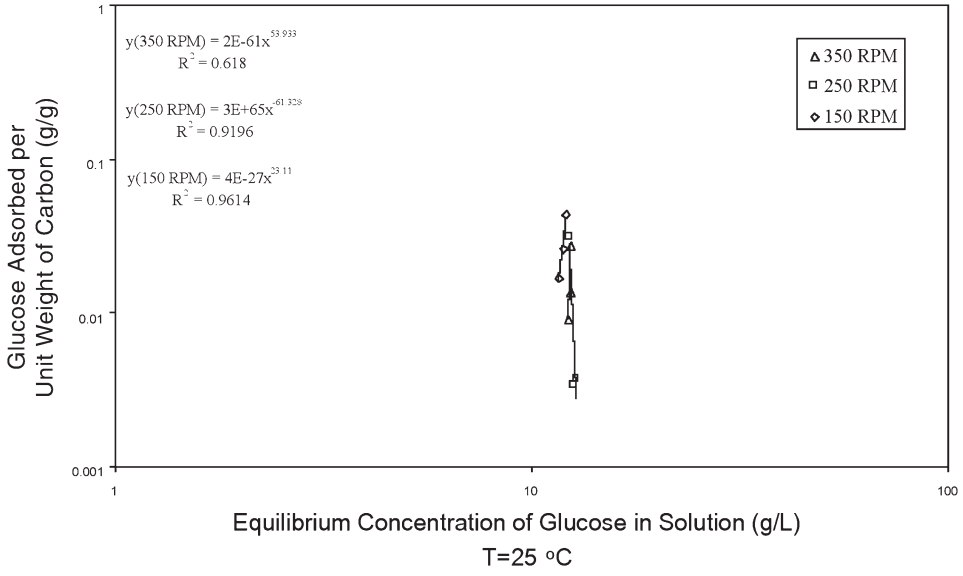

Fig. 4. Adsorption isotherms for glucose.

hydrolysate before enzymatic treatment at 10 to 11 g/L. Final glucose measurements for all conditions tested showed a <1 g/L loss after detoxification. Although xylose is known to be present at much greater concentrations, a xylose-screening method was not available to us at the time of this work. Glucose losses during detoxification are presented here as a starting point for consideration, and future work on the subject should include an investigation into xylose losses.

When no adsorption is desired, an isotherm of a given substance should show a vertical slope. The glucose isotherms in Fig. 4 show vertical slopes, indicating poor adsorption for any amount of carbon loading. The value of equilibrium glucose concentration at the *x*-axis intercept reveals the residual value of glucose in solution. Since increased agitation did not assist glucose adsorption, it is apparent that this choice of carbon powder has the desired characteristic of acetic acid removal with negligible glucose removal. The carbon's affinity for acetic acid and lack of affinity for glucose may be attributed to the size of the molecules. Although the size of the pore opening is unknown, the molecular weight of glucose, 180 g/mol, is three times that of acetic acid, 60 g/mol.

The ultimate goal of detoxification is to reduce the acetic acid concentration below 2 g/L, the level where the inhibitory effects become a factor on fermentation. Since increasing carbon-loading levels increasingly reduces the efficiency of acetic acid removal, it may become impractical to incorporate the amount of carbon necessary to reduce the acetic acid concentration from the initial amount of 16.5 g/L to the desired amount of 2 g/L.

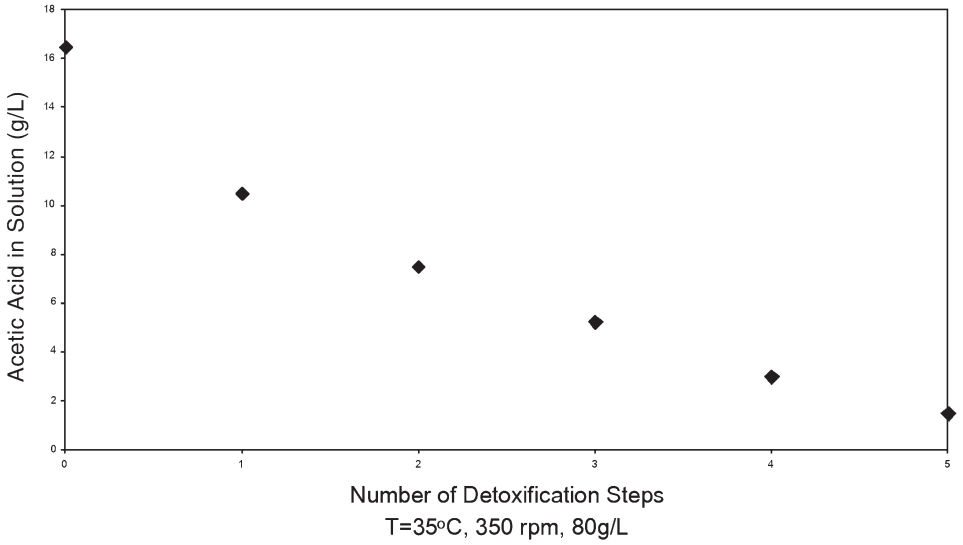

Fig. 5. Multistage detoxification of hydrolysate.

An alternative approach involving a multistage detoxification was tested. After one round of a carbon treatment and subsequent carbon removal process, corn stover solids were added back to the liquid hydrolysate in an amount to yield a final 20% solids concentration that would be used in fermentation. The solids returning to the liquid contained 60% moisture, an amount significant enough to reintroduce acetic acid from the solids to the liquid phase and form a new equilibrium concentration. A second round of carbon treatment and subsequent carbon removal followed. The corn stover solids were removed prior to the reintroduction of carbon so as not to interfere with the contacting of acetic acid with carbon particles. This was repeated until the equilibrium concentration of acetic acid in the liquid phase with solids added back reached 2 g/L or less. Five stages of detoxification were required to lower acetic acid to this level (Fig. 5). Operating conditions for this series of testing were 35°C, 350 rpm, and 80 g/L of carbon.

Although this multistage approach to acetic acid reduction is presented as a feasible option and gives a relative indication of the number of detoxification stages that may be required, any future work on process optimization should also include an investigation of sugar losses incurred over multiple stages.

Conclusion

The same Calgon BL activated carbon that proved effective in removing acetic acid from a synthetic hydrolysate proved somewhat effective in removing acetic acid from actual hydrolysate while glucose adsorption remained minimal. Temperature, between 25 and 35°C, had a minimal

effect on adsorption characteristics. Adsorption of acetic acid was less efficient in the actual hydrolysate than in the synthetic solution. This is attributed to the carbon adsorbing other substances from the hydrolysate. A five-stage detoxification was required to lower acetic acid levels below 2 g/L. Kinetic rate testing yielded a minimum residence time of 5 min for the acetic acid concentration to approach equilibrium between the liquid and carbon phases.

Nomenclature

B = capacity ratio = 1/mK
C_b = bulk reservoir concentration (g/L)
C_{b0} = initial solute concentration (g/L)
D_e = effective diffusivity (m^2/s)
k_f = external mass transfer resistance (m/s)
K = linear isotherm constant
M = mass per bulk volume of carbon (g/L)
R = radius of a particle (m)
T = time (s)
ε = porosity
ρ_s = adsorbent solid density (kg/m^3)
τ = dimensionless time parameter = D_e/R^2

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