DETOXIFICATION AND FERMENTATION OF DISSOLVED COLORED DISCARDED COTTON FABRICS

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Abstract

U.S. Department of Energy has evaluated lignocellulosic biomass as a viable source of material for conversion (i.e., hydrolysis) to simple sugars followed by microbial fermentation to fuels and chemicals. Cotton, in discarded dyed fabric materials, is composed of significant quantities of polymeric sugars in the form of cellulose which can be converted into sugars by the same type of hydrolysis process. However, in addition to the dyes in the discarded cotton products, the conversion process often generates chemical byproducts that are toxic or partially inhibitory to microorganisms. Therefore, our intention was to develop a robust technology that removes these inhibitors prior to fermentation. Results show that powdered activated carbon was effective in removing a significant portion of the dyes (as measured by color reduction) from the hydrolyzed (i.e., solubilized) dyed cotton fabrics; in some cases, 75% was removed. Fermentation of the sugars using two bacterial strains, *Clostridium beijerinckii* for anaerobic fermentation to acetone/butanol/ethanol and *Bacillus subtilis* for aerobic fermentation to acetoin, showed that the activated carbon treatment improved fermentation yields in the case of *C. beijerinckii* over untreated controls. In the case of *B. subtilis*, the results were not as conclusive and suggested that *B. subtilis* may have a higher tolerance to the inhibitors and may not require activated carbons treatment of textile hydrolysates before being used as a carbon source for fermentation.

Introduction

In the U.S., it is estimated that the amount of municipal solid waste related to textiles was 17.03 million tons in 2018, and only 5.73 million tons were recycled or combusted for energy (EPA, 2020). Cotton fiber account for a significant portion of this amount, and while it is fully biodegradable, the presence of dyes makes some fabrics difficult to recycle (Chen and Burns, 2006). The current research project investigated if the cellulose in dyed cotton fabric could be utilized after hydrolysis as a source of sugars in fermentations, and the impact that removal of dyes may have on the fermentation. Fermentation inhibitors are commonly formed during hydrolysis of cellulosic materials under certain conditions ands can be removed by activate carbon treatment (Mussatto and Roberto, 2004).

Acetone and butanol production via fermentation has been used since the First World War (WWI) and commercial plants were constructed for several decades until chemical processes replaced the fermentation process (Jones and Woods 1986). Both acetone and butanol are important industrial solvents and green alternatives for their production are always desirable. Acetoin is a chemical used in food industry and as an important chemical building block; and, while mostly produced by chemical synthesis (Xiao and Lu, 2014), fermentation is cited as a green option (Song et al., 2012).

Materials and Methods

The hydrolysate was created from Reactive Black 5 dyed 100% cotton interlock knit fabric. The method is still proprietary, but in general: The dyed fabric was cut into half-inch wide fabric strips and then cut into smaller pieces. The cotton pieces were given an acidic, high temperature pretreatment. An in-situ buffer was formed after pretreatment to provide the optimal pH prior to addition of cellulase enzyme. A commercial cellulase enzyme cocktail was added to the bath and used for hydrolysis at a constant temperature of 48.9°C for 72 h. The hydrolysate was filtered through Whatman Grade 1 filter paper (pore-size 11 um), and the concentration of glucose in the hydrolysate was 45.4 g/L. The material was stored at -20°C, until used in fermentations.

Two different types of fermentation experiments were conducted (most in triplicate). The first to produce acetone/butanol/ethanol, and the second to produce acetoin. In the first experiments, powdered activated carbon (Filtrasorb 300, Calgon Carbon, Pittsburgh, PA) was mixed with hydrolysate at levels of 0, 0.25, 0.50, 0.75, 1.0,

1.25, 1.50% (w/v), and necessary nutrient salts, minerals, and vitamins (Klasson et al., 2018) for 20-mL fermentations were added. There was no continuous mixing before the mixtures were sterilized via steam sterilization (15 min at 121°C) and color was determined by scanning spectroscopy between 200 and 800 nm at pH 4 and pH 7. Without removing the activated carbon, the mixture was then inoculated with *C. beijerinckii* (NCP 260) for production of acetone/butanol/ethanol as described before (Klasson et al., 2018). Samples were taken of the initial hydrolysate and after addition of the activated carbon and sterilization for determination of color. Samples were also taken after 48 and 72 h of fermentation for analysis of sugars, solvents (e.g., butanol), and organic acids. In the second set of experiments, powdered activated carbon was mixed with hydrolysate at levels of 0, 0.25, 0.50, 0.75, 1.0, 1.25, 1.50% (w/v), and necessary nutrient and supplements (Wright et al., 2020) for 20-mL fermentations were added. Additional glucose was also added to bring the initial glucose concentration up to approximately 65 g/L. The mixtures were sterilized via steam sterilization and color was determined by scanning spectroscopy between 200 and 800 nm at pH 4, pH 7, and pH 9. The mixture was then inoculated with *B. subtilis* (NFRI 8291) for production of acetoin (Wright et al., 2020). Samples were taken of the fermentation medium and then after activated carbon addition and sterilization for determination of color. Samples were also taken after 48 and 72 h of fermentation for analysis of sugars, acetoin, and organic acids.

For each sample, a 2 mL aliquot was drawn from the fermentation culture, centrifuged, and passed through a 0.2 µm syringe filter directly into an HPLC vial which was then fitted with a septum cap. HPLC samples were stored at -20°C prior to analysis. The analyses were carried out by HPLC using 0.6 mL/min 5 mM H_2SO_4 mobile phase, Aminex Cation H guard column, Aminex HPX-87H column (held at 20°C), and diode array and refractive index detectors (Klasson et al., 2018; Wright et al., 2020).

Results and Discussion

Experiments for Acetone/Butanol/Ethanol Production

In the experiments intended to produce acetone/butanol/ethanol, the reduction of color after contact with activated carbon and sterilization was determined by scanning spectroscopy between 200 and 800 nm at pH 4 and pH 7. Measurement could not be done at pH 9, as initially planned, due to continuous gas evolution from the prepared fermentation medium at this pH. The results of the color change are shown Figure 1. The results show that sterilization alone (0%) reduced the absorbance levels (which would be an indication of color intensity reduction). The large band between 470-600 nm in the original sample, is likely related to the reactive dye (De Luca and Nagy, 2020). The results also showed that increasing the activated carbon amount removed more color. There were no significant differences in color measurements at different pH. The purpose of pH adjustment before measurements was that sometimes changes in color for different pH indicate which compounds are causing the color (Godshall, 1997), however, measurements did not vary with pH (Figure 1). In general, the absorbance at a particular wavelength decreases with lower pH, in the case of color associated with sugar degradation; the shoulder around 320 nm (more pronounced at pH 7) in the original sample may be associated with phenolics (Godshall, 1997).

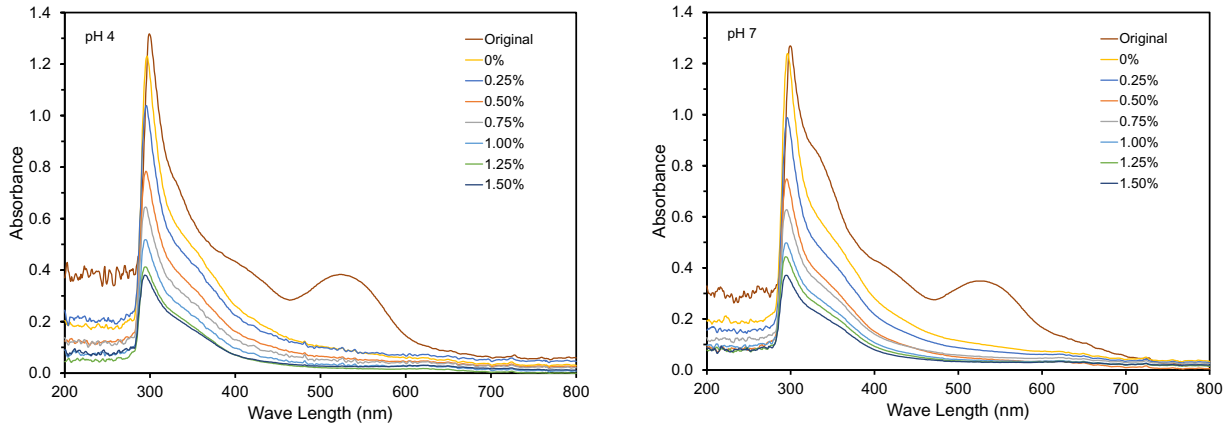

Figure 1. Color reduction of fermentation medium with textile hydrolysate measured at two pH levels (pH 4 and pH 7) after contact with activated carbon at different levels (0-1.50 %).

The presence of activated carbon improved the fermentation to butanol as shown in Figure 2. The total solvent concentration was approximately 11 g/L after 48-72 h for the best fermentations, all of which contained activated carbon. This level of solvent production is slightly below that obtained with glucose in defined medium (Klasson et al., 2018). As all the glucose (see remaining glucose levels in small boxes, Figure 2) was not consumed in these studies, we speculated that the fermentations were nutrient limited.

As salts, minerals, and vitamins were added in all cases, we further speculated that complex nitrogen may be limited and conducted additional experiments with yeast extract amendment. No improvements in glucose consumption were seen (Figure 2, the two columns to the right in each graph), suggested that nutrient limitations were not the cause for incomplete consumption of glucose. Previous experiments with higher levels of glucose in a defined medium produced slightly lower level of solvents but also with unfermented glucose (Klasson et al., 2018).

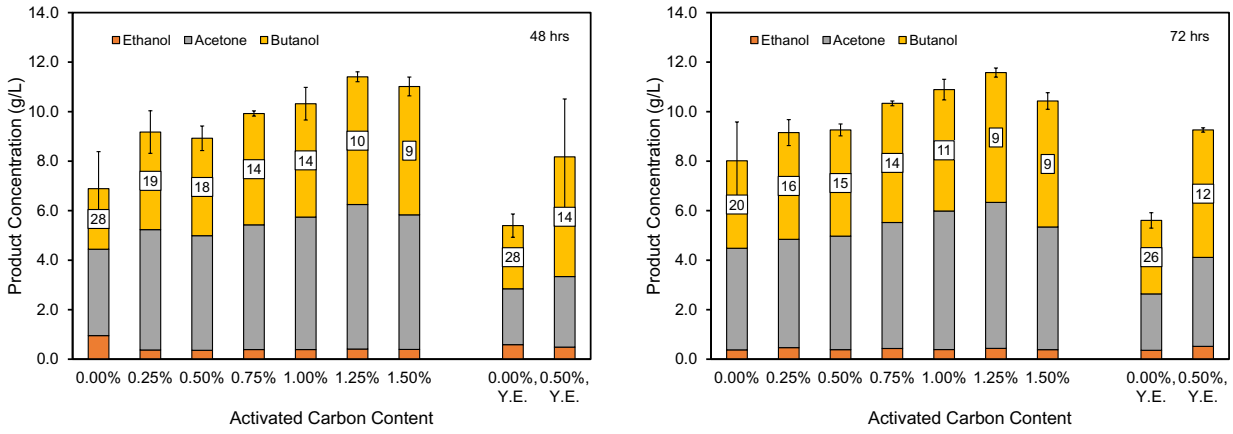

Figure 2. Solvent concentrations after 48 and 72 hours of fermentation of activated carbon treated textile hydrolysate. Two additional experiment were done testing the impact of supplemental yeast extract on the fermentation (two right-most bars in each graph), in which yeast extract was added (1 g/L) with 0 and 0.50% activated carbon. (Error bars are standard error for total solvent concentration from duplicate or triplicate experiments. Values in boxes indicate remaining glucose levels.)

Experiments for Acetoin Production

In the experiments intended to produce acetoin, the reduction of color after contact with activated carbon and sterilization was determined by scanning spectroscopy between 200 and 800 nm at pH 4, pH 7, and pH 9. It should be noted that this fermentation mixture after sterilization without activated carbon (0%) was darker (i.e., more color) than the original hydrolysate (Figure 3). This is because the nutrients added to the hydrolysate contain a very dark component (corn steep liquor). Thus, there was no indication that activated carbon treatment removed color. However, it is possible that the activated carbon removed some toxic dyes from the solubilized fabric.

Main product (acetoin) concentrations during the fermentation are shown in Figure 4. In this case, the presence of activated carbon did not improve the fermentation of glucose to acetoin, conversely it may have hindered acetoin production. Further analysis of the results suggested that acetoin was converted to acetate once most of the glucose was consumed. This indicates that this fermentation must be carefully monitored and halted when the maximum acetoin concentration is achieved. This characteristic (acetoin to acetate conversion) was less prevalent in glucose control experiments. It is unknown why activated carbon appeared to hinder acetoin production and is contrary to previous experiences with this type of fermentation. Possibly, the activated carbon adsorbed the acetoin and prevented its detection (which will be part of a future study). The total acetoin concentration was 24.6 g/L after 72 h for the best fermentations, which did not contain activated carbon. This level was consistent with the glucose control experimental results (right, in Figure 4). All the glucose was consumed in the control studies when the fermentation was allowed to continue for 144 h (data not shown).

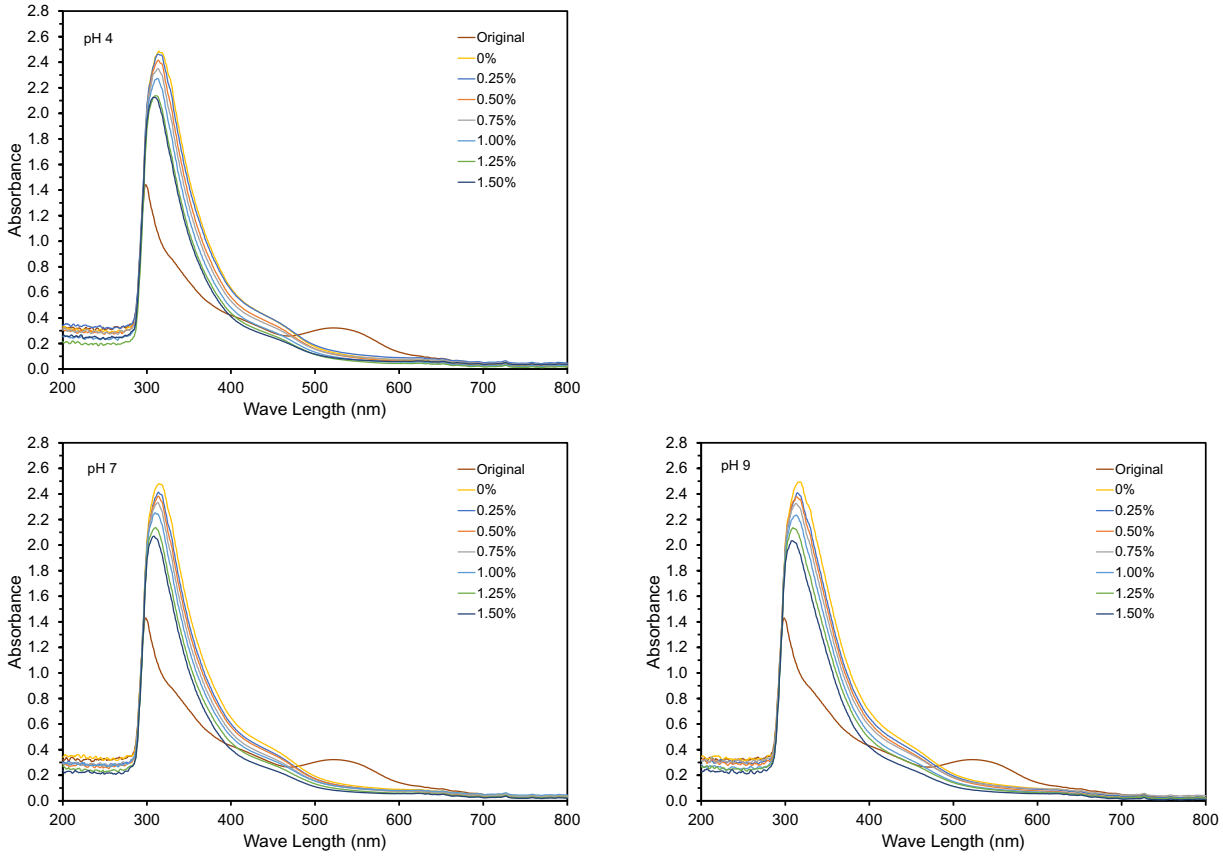

Figure 3. Color reduction of fermentation medium with textile hydrolysate measured at three pH levels (pH 4, pH 7, and pH 9) after contact with activated carbon at different levels (0-1.50 %).

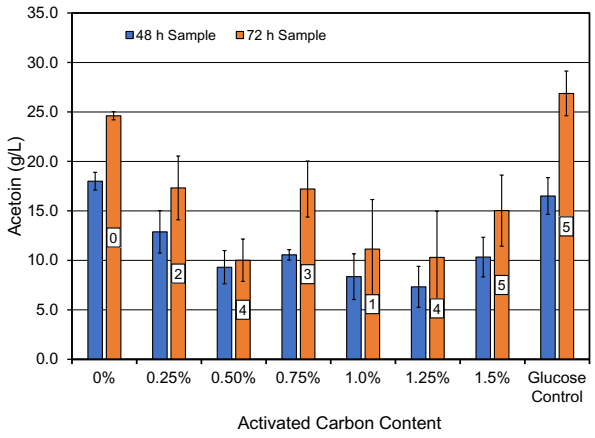

Figure 4. Acetoin concentrations after 48 and 72 hours of fermentation of activated carbon treated textile hydrolysate for different activated carbon levels.

Summary

Powdered activated carbon (PAC) removed color and improved the conversion of glucose from textile hydrolysis to acetone/butanol/ethanol (ABE) using *C. beijerinckii*. This suggests that a toxic material (likely a dye) was removed by PAC. It is suggested that even low levels of PAC are useful for promoting ABE fermentation. PAC was less useful in the fermentation of glucose from textile hydrolyzation to acetoin with *B. subtilis*, where the PAC appeared to inhibit the formation of acetoin (or potentially adsorb the acetoin) and instead caused acetic acid to accumulate. This suggests that PAC is not necessary or helpful in the acetoin fermentation.

Acknowledgements

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