

Pulsed Electric Field Pretreatment of Switchgrass and Wood Chip Species for Biofuel Production

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ABSTRACT: In biomass-to-fuel conversion, the biomass needs to be pretreated so that the cellulose in the plant fibers is exposed for conversion of the lignocellulosic biomass to fuels and chemicals. In this study, we report on the design and fabrication of a pulsed electric field (PEF) system for pretreatment of wood chip and switchgrass samples. Wood chip samples were given 1000 and 2000 pulses of 1 kV/cm, and 1000, 2000, and 5000 pulses of 10 kV/cm with a pulse width of 100 μ s and frequency of 3 Hz. Switchgrass samples were given 1000, 2000, and 5000 pulses of 2.5, 5, 8, and 10 kV/cm with a pulse width of 100 μ s and frequency of 3 Hz. The uptake of a colored dye neutral red C₁₅H₁₇ClN₄ ($M_w \sim 289$) in untreated and PEF treated samples was studied to quantify the effect of PEF treatment on diffusion in plant tissues. Wood chip samples treated at 1 kV/cm showed a dye uptake similar to that of untreated wood chip samples. The switchgrass samples were resistant to change in the structure at low field strengths up to 5 kV/cm. The samples treated at field strengths of ≥ 8 kV/cm showed faster and larger neutral red uptake, suggesting an increase in the porosity of switchgrass samples. Similar phenomena were observed for wood chip samples treated at 10 kV/cm. Permeabilization of switchgrass and wood chip species using PEF can be utilized to improve cellulose hydrolysis to sugar and hence efficient fuel conversion.

1. INTRODUCTION

With rapid increase in oil prices over the past decade and concerns about future oil supplies, alternative sources of liquid fuels have recently gained extensive attention.¹ Burning fossil fuels such as coal and oil releases CO₂, which is a major cause of global warming.² Conversion of abundant lignocellulosic biomass to fuels and chemicals presents a viable option for energy security and reduction of greenhouse gas emissions.³ Agricultural residues such as wheat straw and corn stover, forest products (e.g., hardwood and softwood), and dedicated crops such as switchgrass and salix are examples of lignocellulosic biomasses which can be used as renewable sources of energy.⁴ The lignocellulosic biomasses are produced from plants grown today, and combusting them can maintain a constant carbon cycle unlike burning fossil fuels which come from plants that grew millions of years ago.⁴ Cellulosic ethanol and ethanol produced from other biomass resources provide a possibility to cut greenhouse gas emissions by 86%.⁵

Approximately 90% of the dry weight of most plant materials is stored in the form of cellulose, hemicellulose, lignin, and pectin.² Plant cell structure is composed of the primary cell wall, secondary cell wall, and cell membrane.⁴ The primary cell wall consists of cellulose, hemicellulose, and pectin. A schematic for conversion of biomass to fuel is shown in Figure 1. The biomass to fuel conversion process includes hydrolysis of various components in the lignocellulosic materials to fermentable reducing sugars and the subsequent fermentation of the sugars to fuels such as ethanol and butanol.^{4,6} The presence of lignin in lignocelluloses leads to a protective barrier that prevents plant cell destruction by fungi and bacteria for conversion to fuel. For biomass to fuel conversion, the cellulose and hemicellulose must be broken down to their corresponding monomers (sugars) so

that microorganisms can utilize them. Pretreatment is mainly required for efficient hydrolysis of cellulose to its constituent sugars.

The digestibility of cellulose present in lignocellulosic biomass is hindered by many physicochemical, structural, and compositional factors. In lignocellulosic biomass to fuel conversion, the biomass needs to be pretreated so that the cellulose in the plant fibers is exposed. The factors affecting the hydrolysis of cellulose include porosity (accessible surface area) of the biomass materials, cellulose fiber crystallinity, and content of both lignin and hemicellulose.⁷ The presence of lignin and hemicellulose makes the accessibility of cellulase enzymes and acids to cellulose more difficult, thus reducing the efficiency of the hydrolysis process. Pretreatment is required to alter the size and structure of the biomass, as well as its chemical composition. The hydrolysis process can be significantly improved by removal of lignin and hemicellulose, reduction of cellulose crystallinity, and increase of porosity through the pretreatment processes.⁷

Various methods are used for pretreatment of biomass.^{8–28} These methods are divided into following categories: physical (milling and grinding),^{8–10} physicochemical (steam pretreatment/ autohydrolysis, ammonia fiber explosion (AFEX), hydrothermolysis, and wet oxidation),^{11–17} chemical (alkali, dilute acid, oxidizing agents, and organic solvents),^{18–25} biological,^{26–28} or a combination of these. Details of these methods along with their advantages and limitations have been discussed in detail in our previously published review article.⁴ In this study, we report a

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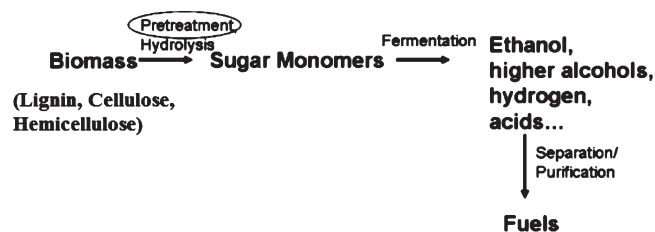


Figure 1. Schematic of conversion of lignocellulosic biomass to fuel.

novel pulsed electric field (PEF) pretreatment of biomass. To our knowledge, no studies have been reported for pretreatment of biomass using PEF. Pulsed electrical field (PEF) involves application of a very short burst ($\sim 100 \mu\text{s}$) of high voltage to a sample placed between two electrodes. Application of a high-intensity external electric field leads to an induction of critical electric potential across the cell membrane, which leads to rapid electrical breakdown and local structural changes of the cell membrane and the cell wall and therefore plant tissue. The electric field results in a dramatic increase in mass permeability and, in some cases, mechanical rupture of the plant tissue.⁴ Based on this phenomenon, many practical applications of high electric fields for reversible or irreversible permeabilization of various biological systems have been studied in the fields of medicine and bioscience.^{29,30} The permeabilization of plant membranes to improve mass transfer of metabolites is currently of interest to the food industry. Recently there have also been reports on the application of PEF for inactivation of enzymes.³¹ Pulsed electrical fields have also been applied on vegetable tissues to improve mass transfer operations such as diffusion of soluble substances,³² juice extraction,^{33,34} and dehydration processes.³⁵ By application of high field strengths in the range 5–20 kV/cm, plant cells can be significantly ruptured. By applying electric pulses of high field strengths, PEF can create damage in the plant tissue and hence facilitate the entry of acids or enzymes used to break down the cellulose into its constituent sugars. In the case of chemical modification of plant tissue, particularly in lignocellulose hydrolysis, appropriate chemicals may need to be transported into the tissue to aid in cell wall breakdown and digestion, and pretreatment with PEF is important to facilitate the process. In this paper, we report PEF treatment of dried plant tissues such as switchgrass and wood chips for which the cell membranes are already destroyed^{36,37} and therefore PEF will not affect the cell membranes. However, PEF also leads to destruction of the plant cell wall and enzymes. An advantage of PEF is that it can be carried out at ambient conditions and energy use is low because pulse times are very short ($100 \mu\text{s}$). Thus a large number of pulses can be applied over a relatively short time. Furthermore, the actual PEF process itself does not involve moving parts, so the equipment is not complex. PEF treatment has inherent advantages, which include low energy and processing at normal temperatures and pressure.

We report the application of PEF for pretreatment of the lignocellulosic biomass species wood chips and switchgrass. Wood chips and switchgrass samples were treated with PEF with field strengths up to 10 kV/cm, and neutral red dye uptake experiments were used to characterize the extent of permeabilization of the biomass after application of PEF.

2. EXPERIMENTAL SECTION

We designed and fabricated a system for PEF treatment of biomass feedstocks. A schematic of the PEF system is shown in

Figure 2. The PEF system consists of a high-voltage power supply, a pulse/function generator (HP811A pulse/function generator), a switching circuit, and a sample holder. The switching circuit consists of a MOSFET transistor which is driven by the function generator, a capacitor, and resistors. The function generator feeds the pulse of desired frequency and duty cycle to the switching circuit. The switching circuit is turned on when the pulse is applied to it and transfers the high voltage supplied by the power supply across the sample holder. Consequently, high-voltage pulses of square shape can be applied to the sample using the function generator and switching circuit.

Wood chips (Southern pine) samples were obtained from Chevron Energy, Houston, TX, and switchgrass samples were provided by the Plant Sciences Department at the University of California, Davis. The wood chip samples of particle size ~ 1.5 mm were used as obtained. The switchgrass samples were cut into small pieces and then sieved using USDA sieves to obtain switchgrass samples of particle size between 0.85 and 1.65 mm. Prior to the PEF experiments the samples were suspended in distilled water. The PEF treatment was done by placing concentrated wood chips in water suspension (or switchgrass) in the sample holder and then applying the high-voltage pulses. Wood chip samples were given 1000 and 2000 pulses of 1 kV/cm and 1000, 2000, and 5000 pulses of 10 kV/cm with a pulse width of $100 \mu\text{s}$ and frequency of 3 Hz. Switchgrass samples were given 1000, 2000, and 5000 pulses of 2.5, 5, 8, and 10 kV/cm with a pulse width of $100 \mu\text{s}$ and frequency of 3 Hz.

The uptake of a colored dye neutral red $\text{C}_{15}\text{H}_{17}\text{ClN}_4$, $M_W = 289$ (Sigma Aldrich), on the untreated and PEF treated samples was studied to quantify the effect of PEF treatments on diffusion into the switchgrass and wood chip samples. The samples were rapidly stirred (400 rpm) in an aqueous solution of neutral red, and the concentration of dye in water was measured as a function of time using a Carey (Model 3) UV–visible spectrophotometer. A calibration curve was first prepared by using known concentrations of dye in water and obtaining the corresponding absorbance at 550 nm. The calibration curve was used to calculate the dye concentration for any absorbance value. The dye uptake rate in the biomass samples was calculated by determining the decrease of the dye concentration in the aqueous solution as a function of time and then performing a dye mass balance. Thus the effect of PEF treatment on diffusion of dye into the sample could be elucidated.

The apparent distribution coefficients of dye in untreated and PEF treated biomass samples were calculated to quantify the effect of PEF treatment. We use the term “apparent distribution coefficient” because the uptake of dye into the wetted biomass is complex and the biomass is not a homogeneous phase. A mass balance for dye was made to obtain the apparent distribution coefficient according to the following equations:

$$C_0 V_0 = C V_0 + C_{\text{sample}} V_{\text{sample}}$$

$$V_0 = \left(\frac{C}{C_0} \right) V_0 + \left(\frac{C_{\text{sample}}}{C_0} \right) V_{\text{sample}}$$

$$V_0 = \left(\frac{C}{C_0} \right) V_0 + K_{\text{distribution}} V_{\text{sample}}$$

where C_0 is the initial dye concentration, C is the final dye concentration, V_0 is the volume of the solution, and V_{sample} is the volume of sample.

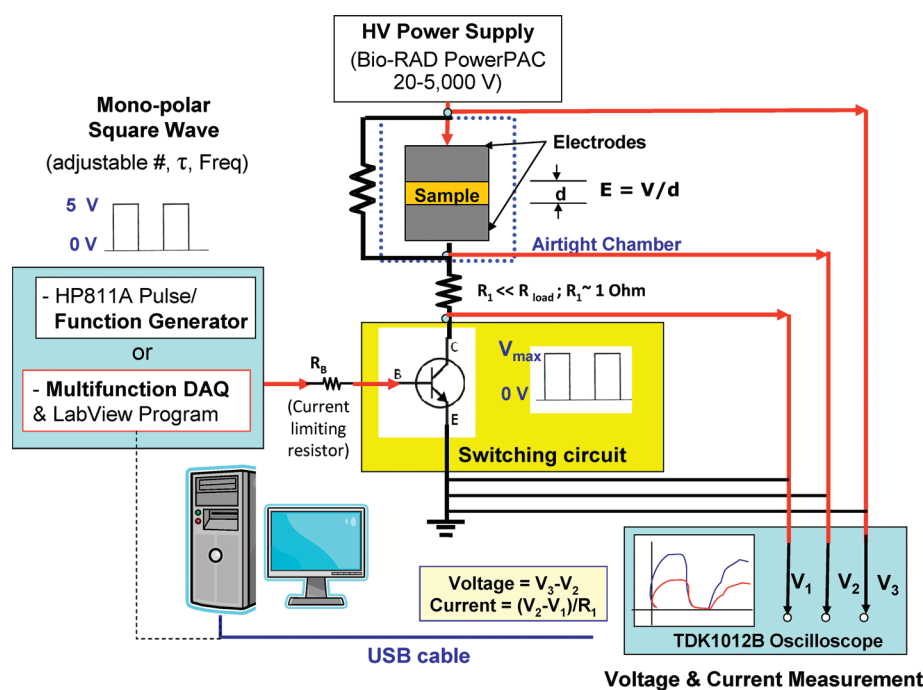


Figure 2. PEF system for pretreatment of biomass feedstocks. The sample itself is a resistor and is shown schematically.

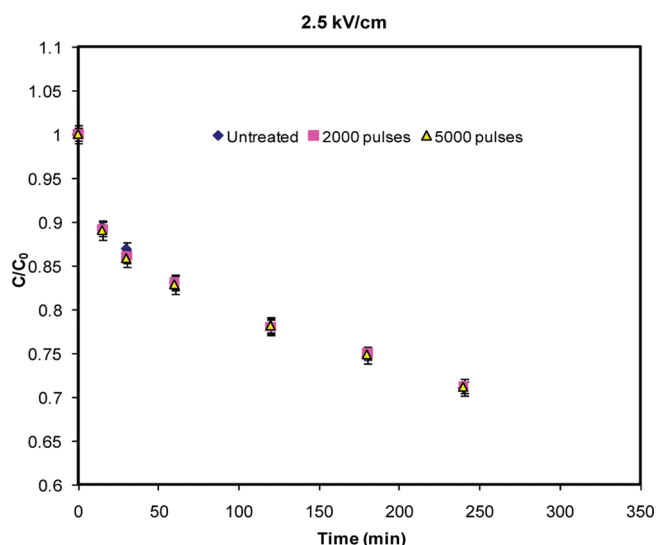


Figure 3. Neutral red dye (NR) uptake experiments for untreated and PEF treated switchgrass samples at 2.5 kV/cm.

3. RESULTS AND DISCUSSION

For the dye uptake experiment, 0.02 g of untreated and PEF treated biomass samples was stirred in the aqueous neutral red solution. The initial concentration of the dye solution was obtained from the calibration curve by obtaining the absorbance versus concentration curve using the UV-vis spectrophotometer. The concentration of aqueous neutral red solution was set to obtain an initial solution absorbance below 1. Absorbances of neutral red solution were then obtained as a function of time and converted to concentration using the calibration curve. Change in neutral red concentration of the solution was plotted against time for the duration of the uptake experiment to quantify

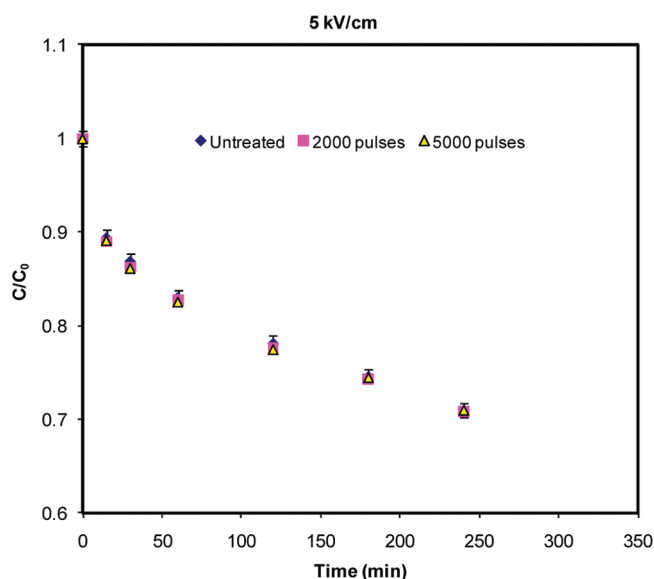


Figure 4. Neutral red dye (NR) uptake experiments for untreated and PEF treated (5 kV/cm) switchgrass samples.

the effect of the PEF treatment. This experiment was performed to study the generation of porosity in the samples due to the use of PEF. As a result of creation of porosity due to PEF treatment of biomass materials, the rate of dye uptake in the biomass samples should be enhanced as the dye can penetrate the biomass samples through the pores.

Dye uptake experiment results for switchgrass samples treated at 2.5 kV/cm, in comparison with untreated switchgrass samples, are shown in Figure 3. The switchgrass samples treated at 2.5 kV/cm for both 2000 and 5000 pulses show dye uptake characteristics similar to those of untreated samples. Thus PEF treatment

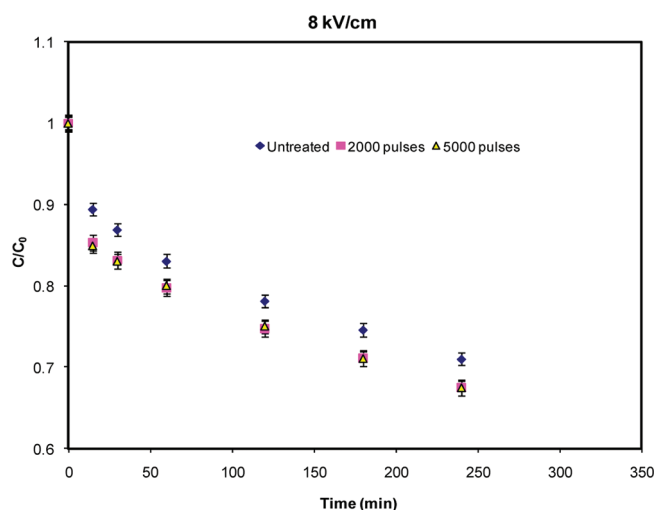


Figure 5. Neutral red dye (NR) uptake experiments for untreated and PEF treated (8 kV/cm) switchgrass samples.

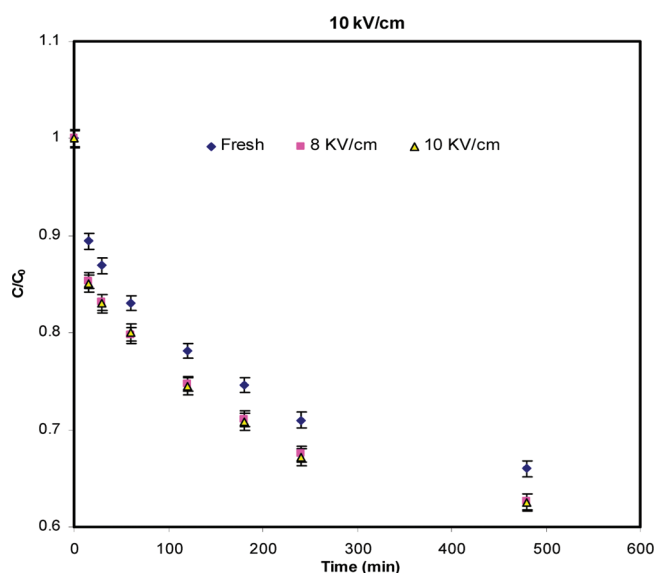


Figure 6. Neutral red dye (NR) uptake experiments for untreated (fresh) switchgrass samples and PEF treated switchgrass samples at 8 and 10 kV/cm for 2000 pulses.

at 2.5 kV/cm has no positive effect on increasing the permeability and therefore mass transfer into the switchgrass samples. The results of dye uptake experiments for switchgrass samples treated at 5 kV/cm, in comparison with untreated switchgrass samples, are shown in Figure 4. The switchgrass samples treated at 5 kV/cm for 2000 and 5000 pulses also show dye uptake characteristics similar to those of untreated samples. Thus treatment of switchgrass samples with PEF at 5 kV/cm does not lead to any apparent alteration in the sample structure.

The dye uptake comparison for switchgrass samples treated at 8 kV/cm to untreated switchgrass samples is shown in Figure 5. The switchgrass samples treated at 8 kV/cm show faster and higher dye uptake compared to the untreated samples, suggesting an alteration in the switchgrass permeability. The switchgrass samples treated at 8 kV/cm with 2000 and 5000 pulses show similar neutral red uptake characteristics, suggesting that 2000 pulses were

Table 1. Apparent Distribution Coefficient Comparison for Untreated and Treated Switchgrass Samples

sample	$(C/C_0)_{\text{final}}$	$K_{\text{distribution}}$
untreated	0.56	1050
2.5 kV/cm, 2000 pulses	0.56	1050
2.5 kV/cm, 5000 pulses	0.56	1050
5 kV/cm, 2000 pulses	0.56	1050
5 kV/cm, 5000 pulses	0.56	1050
8 kV/cm, 2000 pulses	0.525	1200
8 kV/cm, 5000 pulses	0.52	1200
10 kV/cm, 2000 pulses	0.52	1200

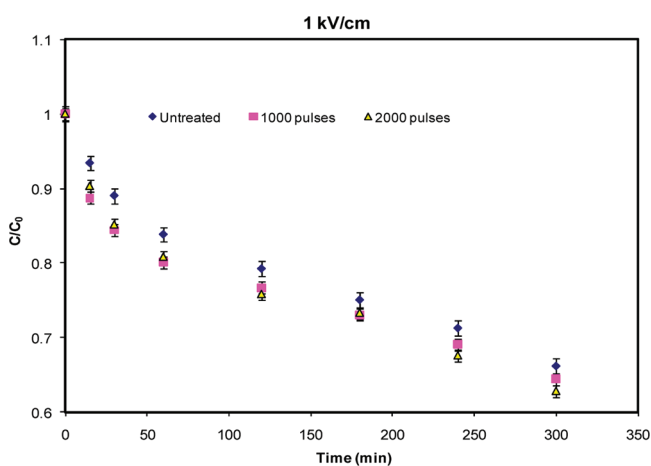


Figure 7. Neutral red dye (NR) uptake experiments for untreated wood and PEF treated samples at 1 kV/cm.

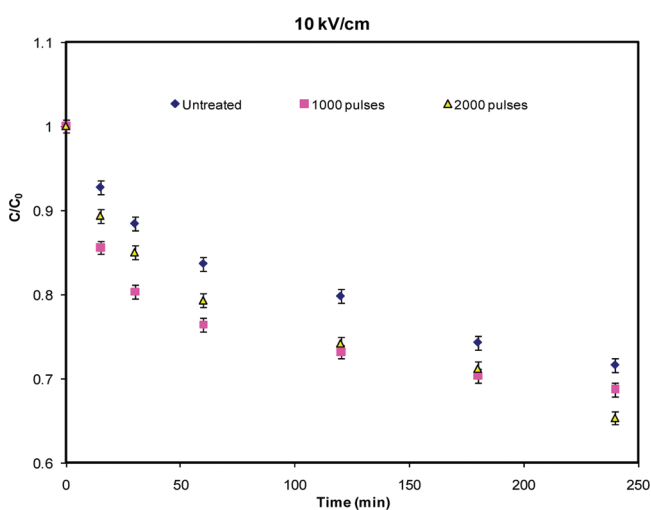


Figure 8. Neutral red dye (NR) uptake experiments for untreated (fresh) wood and PEF treated samples at 10 kV/cm.

sufficient to generate porosity with PEF treatment of the switchgrass samples.

The switchgrass samples were then treated with PEF at 10 kV/cm with 2000 pulses, and comparisons were made for the neutral red uptake of untreated, samples treated at 8 and 10 kV/cm. The

Table 2. Apparent Distribution Coefficient Comparison for Untreated and Treated Wood Chip Samples

sample	$(C/C_0)_{\text{final}}$	$K_{\text{distribution}}$
untreated	0.55	1300
1 kV/cm, 1000 pulses	0.55	1300
1 kV/cm, 2000 pulses	0.545	1325
10 kV/cm, 2000 pulses	0.54	1350
10 kV/cm, 2000 pulses	0.535	1400

results of these experiments for samples treated with 2000 pulses are shown in Figure 6. The PEF samples treated at 10 kV/cm show neutral red uptake characteristics similar to those of samples treated at 8 kV/cm.

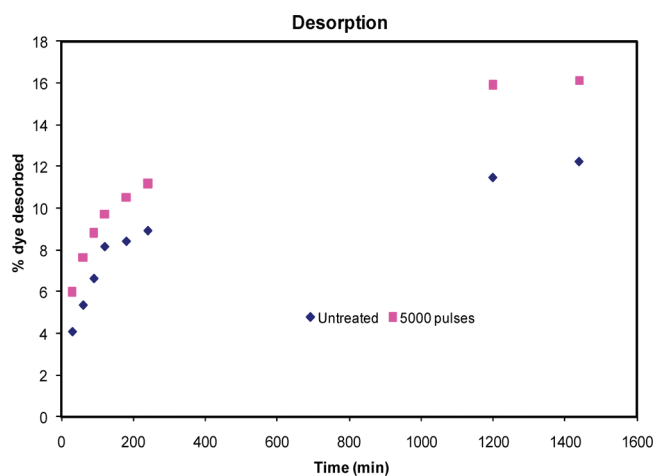
The apparent distribution coefficient reflects the ratio of dye concentration in the switchgrass sample to the concentration of dye in the aqueous phase. High values of the apparent distribution coefficient suggest that the neutral red dye has the preference to stay in the biomass phase compared to the aqueous phase, which should be facilitated by permeabilization and hence an increase in the porosity of biomass samples. The values of the apparent distribution coefficients for the untreated and treated switchgrass samples are shown in Table 1. The switchgrass samples treated at field strengths of ≥ 8 kV/cm have apparent distribution coefficients for neutral red higher than the untreated switchgrass samples, indicating an increase in the porosity of switchgrass.

The results of neutral red uptake experiments for wood chip samples treated at 1 kV/cm in comparison with untreated wood chip samples are shown in Figure 7. The PEF treated wood chip samples show dye uptake characteristics similar to those of untreated samples, indicating negligible effect of PEF on the wood chip samples for the 1 kV/cm condition. The results of dye uptake experiments for wood chip samples treated at 10 kV/cm in comparison with untreated samples are shown in Figure 8. The wood chip samples treated at 10 kV/cm show faster and higher dye uptake compared to untreated samples, suggesting an alteration in the porosity of the wood chips.

Apparent distribution coefficients of dye in untreated and treated wood chip samples were calculated to quantify the effect of PEF treatment on the samples. The values of the apparent distribution coefficients for the untreated and treated wood chip samples are shown in Table 2. The apparent distribution coefficients for neutral red dye for wood chip samples treated at field strength of 10 kV/cm have higher values than those for the untreated wood chip samples. The apparent distribution coefficient of the dye is very high, indicating that much of the dye is bound to the rehydrated biomass.

A comparison of the increase in the apparent distribution coefficient of switchgrass and wood chip samples treated at 10 kV/cm with 2000 pulses was made. An increase in distribution coefficient of $\sim 14.5\%$ is observed for switchgrass samples compared to $\sim 7\%$ for wood chip samples. This suggests that switchgrass samples were easier to electroporate compared to wood chip samples. Wood chips typically have a lignin content of $\sim 25\%$, which is higher than the lignin content of switchgrass at $\sim 12\%$.⁴ Lignin is extremely difficult to break down, and this fact may be a reason for the lower electroporation of wood chips compared to switchgrass.

After the dye uptake experiment, desorption of neutral red was obtained by stirring the switchgrass samples obtained after dye

**Figure 9.** Neutral red desorption characteristics of untreated (fresh) and PEF treated samples at 8 kV/cm switchgrass samples.

uptake experiments in pure deionized water. The desorption characteristics of neutral red from untreated and PEF treated switchgrass samples are shown in Figure 9. Desorption of neutral red from the PEF treated sample was faster than that from the untreated switchgrass sample. Further, a very low amount of dye was desorbed, suggesting that much of the dye is irreversibly bound on sites in the switchgrass samples, possibly due to electrostatic interaction and chemisorption.

4. CONCLUSIONS

A pulsed electric field (PEF) system for treatment of switchgrass and wood chip samples was designed and fabricated. The PEF system was used to treat the switchgrass and wood chip samples up to field strengths of 10 kV/cm. The switchgrass samples are resistant to any change in the structure at low field strengths up to 5 kV/cm. The samples treated at field strengths of ≥ 8 kV/cm show faster and higher neutral red dye uptake, suggesting an increase in the porosity of switchgrass samples. The neutral red dye appears to be in part irreversibly bound in the samples. Thus the apparent distribution coefficient of the dye is very high.

Similar phenomena are observed for wood chip samples treated at 10 kV/cm. Wood chip samples treated at 1 kV/cm show dye uptake similar to that of untreated wood chip samples. However, 2000 pulses are sufficient to cause a change in the dye uptake by the wood biomass samples at 10 kV/cm field strength. Samples treated at a higher number of pulses show neutral red uptake similar to that of samples treated with 2000 pulses.

Increase in porosity of biomass may be useful in increasing the enzyme hydrolysis rates or acid hydrolysis of biomass samples. Therefore, PEF can be a promising technology for improvement of pretreatment of lignocellulosic biomass for biofuel production.

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■ REFERENCES

- (1) Prior, B. A.; Day, D. F. Hydrolysis of ammonia-pretreated sugar cane bagasse with Cellulase, β -Glucosidase, and hemicellulase preparations. *Appl. Biochem. Biotechnol.* **2008**, *146*, 151–164.
- (2) Yat, S. C.; Berger, A.; Shonnard, D. R. Kinetic characterization of dilute surface acid hydrolysis of timber varieties and switchgrass. *Bioresour. Technol.* **2008**, *99*, 3855–3863.
- (3) Wyman, C. E. Biomass ethanol: technical progress, opportunities, and commercial challenges. *Annu. Rev. Energy Environ.* **1999**, *24*, 189–226.
- (4) Kumar, P.; Barrett, D. M.; Delwiche, M. J.; Stroeve, P. Methods for pretreatment of lignocellulosic biomass for efficient hydrolysis and biofuel production. *Ind. Eng. Chem. Res.* **2009**, *48* (8), 3713–3729.
- (5) Baral, A.; Bakshi, B. R. Comprehensive study of cellulosic ethanol using hybrid eco-LCA. In *Bioenergy and Biofuel from Biowastes and Biomass*; Khanal, S. K., Surampalli, R. Y., Zhang, T. C., Lamsal, B. P., Tyagi, R. D., Eds.; ASCE Publications: Reston, VA, 2010; pp 434–456.
- (6) Yang, Y.; Sharma-Shivappa, R. R.; Burns, J. C.; Cheng, J. Saccharification and Fermentation of Dilute-Acid-Pretreated Freeze-Dried Switchgrass. *Energy Fuels* **2009**, *23*, 5626–5635.
- (7) McMillan, J. D. Pretreatment of lignocellulosic biomass. In *Enzymatic Conversion of Biomass for Fuels Production*; Himmel, M. E., Baker, J. O., Overend, R. P., Eds.; American Chemical Society: Washington, DC, 1994; pp 292–324.
- (8) Sun, Y.; Cheng, J. Hydrolysis of lignocellulosic materials for ethanol production: a review. *Bioresour. Technol.* **2002**, *83*, 1–11.
- (9) Millet, M. A.; Baker, A. J.; Scatter, L. D. Physical and chemical pretreatment for enhancing cellulose saccharification. *Biotechnol. Bioeng. Symp.* **1976**, *6*, 125–153.
- (10) Cadoche, L.; Lopez, G. D. Assessment of size reduction as a preliminary step in the production of ethanol from lignocellulosic wastes. *Biol. Wastes* **1989**, *30*, 153–157.
- (11) Li, J.; Henriksson, G.; Gellerstedt, G. Lignin depolymerization/repolymerization and its critical role for delignification of aspen wood by steam explosion. *Bioresour. Technol.* **2007**, *98*, 3061–3068.
- (12) Kabel, M. A.; Bos, G.; Zeevalking, J.; Voragen, A. G.; Schols, H. A. Effect of pretreatment severity on xylan solubility and enzymatic breakdown of the remaining cellulose from wheat straw. *Bioresour. Technol.* **2007**, *98*, 2034–2042.
- (13) Alizadeh, H.; Teymouri, F.; Gilbert, T. I.; Dale, B. E. Pretreatment of switchgrass by ammonia fiber explosion (AFEX). *Appl. Biochem. Biotechnol.* **2005**, *121*–123, 1133–1141.
- (14) Murnen, H. K.; Balan, V.; Chundawat, S. P. S.; Bals, B.; Sousa, L. D. C.; Dale, B. E. Optimization of ammonia fiber expansion (AFEX) pretreatment and enzymatic hydrolysis of miscanthus \times giganteus to fermentable sugars. *Biotechnol. Prog.* **2007**, *23*, 846–850.
- (15) Isci, A.; Himmelsbach, J. N.; Pometto, A. L.; Raman, R.; Anex, R. P. Aqueous ammonia soaking of switchgrass followed by simultaneous saccharification and fermentation. *Appl. Biochem. Biotechnol.* **2008**, *144*, 69–77.
- (16) Kim, T. H.; Lee, Y. Y. Pretreatment and fractionation of corn stover by ammonia recycle percolation process. *Bioresour. Technol.* **2005**, *96*, 2007–2013.
- (17) Zheng, Y. Z.; Lin, H. M.; Tsao, G. T. Pretreatment for cellulose hydrolysis by carbon dioxide explosion. *Biotechnol. Prog.* **1998**, *14*, 890–896.
- (18) Quesada, J.; Rubio, M.; Gomez, D. Ozonation of Lignin Rich Solid Fractions from Corn Stalks. *J. Wood Chem. Technol.* **1999**, *19*:1, 115–137.
- (19) Esteghlalian, A.; Hashimoto, A. G.; Fenske, J. J.; Penner, M. H. Modeling and optimization of the dilute-sulfuric-acid pretreatment of corn stover, poplar and switchgrass. *Bioresour. Technol.* **1997**, *59*, 129–136.
- (20) Ishizawa, C. I.; Davis, M. F.; Schell, D. F.; Hohnson, D. K. Porosity and its effect on the digestibility of dilute sulfuric acid pretreated corn stover. *J. Agric. Food Chem.* **2007**, *55*, 2575–2581.
- (21) Kim, S.; Holtzaple, M. T. Effect of structural features on enzyme digestibility of corn stover. *Bioresour. Technol.* **2006**, *97*, 583–591.
- (22) Karr, W. E.; Holtzaple, T. Using lime pretreatment to facilitate the enzymatic hydrolysis of corn stover. *Biomass Bioenergy* **2000**, *18*, 189–199.
- (23) Hu, Z.; Wen, Z. Enhancing enzymatic digestibility of switchgrass by microwave-assisted alkali pretreatment. *Biochem. Eng. J.* **2008**, *38*, 369–378.
- (24) Arato, C.; Pye, E. K.; Gjennestad, G. The lignol approach to biorefining of woody biomass to produce ethanol and chemicals. *Appl. Biochem. Biotechnol.* **2005**, *121*–124, 871–882.
- (25) Pan, X.; Gilkes, N.; Kadla, J.; Pye, K.; Saka, H.; Gregg, D.; Ehara, K.; Xie, D.; Lam, D.; Saddler, J. Bioconversion of hybrid poplar to ethanol and co-products using an organosolv fractionation process: Optimization of process yields. *Biotechnol. Bioeng.* **2006**, *94* (5), 851–861.
- (26) Okano, K.; Kitagawa, M.; Sasaki, Y.; Watanabe, T. Conversion of Japanese red cedar (*Cryptomeria japonica*) into a feed for ruminants by white-rot basidiomycetes. *Anim. Feed Sci. Technol.* **2005**, *120*, 235–243.
- (27) Lee, J.-W.; Gwak, K.-S.; Park, J.-Y.; Park, M.-J.; Choi, D.-H.; Kwon, M.; Choi, I.-G. Biological pretreatment of softwood pinus densiflora by three white rot fungi. *J. Microbiol.* **2007**, *45* (6), 485–491.
- (28) Singh, P.; Suman, A.; Tiwari, P.; Arya, N.; Gaur, A.; Shrivastava, A. K. Biological pretreatment of sugarcane trash for its conversion to fermentable sugars. *World J. Microbiol. Biotechnol.* **2008**, *24*, 667–673.
- (29) Knorr, D.; Angersbach, A. Impact of high-intensity electrical field pulses on plant membrane permeabilization. *Trends Food Sci. Technol.* **1998**, *9*, 185–191.
- (30) Angerbasch, A.; Heinz, V.; Knorr, D. Effects of pulsed electric fields on cell membranes in real food systems. *Innovative Food Sci. Emerging Technol.* **2000**, *1*, 135–149.
- (31) Giner, G.; Gimeno, V.; Barbosa-Canovas, G. V.; Martin, O. Effects of pulsed electric field processing on apples and pear polyphenoloxidases. *Food Sci. Technol. Int.* **2001**, *7* (4), 339–345.
- (32) Taiwo, K. A.; Angerbasch, A.; Ade-Omowaye, B. I. O.; Knorr, D. Effects of pretreatments on the diffusion kinetics and some quality parameters of osmotically dehydrated apple slices. *J. Agric. Food Chem.* **2001**, *49* (6), 2804–2811.
- (33) Eshtiaghi, M. N.; Knorr, D. High electric field pulse treatment: potential for sugar beet processing. *J. Food. Eng.* **2002**, *52* (3), 265–272.
- (34) Bazhal, M. I.; Lebvoka, N. I.; Vorobiev, E. Pulsed electric field treatment of apple tissue during compression for juice extraction. *J. Food. Eng.* **2001**, *50* (3), 129–139.
- (35) Rastogi, N. K.; Eshtiaghi, M. N.; Knorr, D. Accelerated mass transfer during osmotic dehydration of high intensity electric field pulse treated potatoes. *J. Food Sci.* **1999**, *64* (6), 1020–1023.
- (36) Prothon, F.; Ahne, L.; Sjolholm, I. Mechanisms and prevention of plant tissue collapse during dehydration: A Critical Review. *Crit. Rev. Food Sci. Nutr.* **2003**, *43* (4), 447–479.
- (37) Hills, B. P.; Remigereau, B. NMR studies of changes in sub-cellular water compartmentation in parenchyma apple tissue during drying and freezing. *Int. J. Food Sci. Technol.* **1997**, *32* (1), 51–61.