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Pulsed Electric Field-Based Pretreatment of Flax Straw Influence of Particle Size on Efficiency

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Abstract:

Biofuels are emerging as a sustainable and eco-friendly source of energy, primarily derived from renewable and low-cost feedstocks such as lignocellulosic biomass. However, the efficiency of biofuel production from lignocellulose is hindered by the complex composition and structural characteristics of the biomass, particularly the high cellulose crystallinity and the presence of lignin. These factors contribute to poor hydrolysis rates, resulting in low yields of fermentable sugars, which are essential for biofuel production. To overcome this challenge, a pretreatment step is often incorporated to enhance the digestibility of lignocellulosic biomass by reducing lignin content and cellulose crystallinity, thereby facilitating more efficient saccharification.

Among the various pretreatment technologies, Pulsed Electric Field (PEF) has shown promising results in improving the permeability of plant tissues. This process involves the application of high-voltage electric pulses, which create temporary pores in the cell membranes, enhancing the access of enzymes during subsequent hydrolysis. In this study, flax straw, a readily available lignocellulosic biomass, was subjected to PEF pretreatment. The hydrolysis of pretreated flax straw led to significant improvements in the yields of fermentable sugars, including glucose, cellobiose, and xylose. Specifically, the yields of glucose, cellobiose, and xylose were increased by 25%, 67%, and 12%, respectively, compared to untreated flax straw. These results demonstrate the potential of PEF as an effective pretreatment method to enhance the saccharification process, thereby improving the overall efficiency of biofuel production from lignocellulosic biomass.

Keywords: Biofuel; Biorefinery; Flax straw; Lignocellulose biomass; Pretreatment; Pulsed electric field; Saccharification

1. Introduction

In response to the growing need for alternative energy sources with lower environmental impact, a variety of renewable energy options have been explored, including nuclear energy, solar power, wind energy, biofuels, and coal/gas-to-liquid technologies. Among these, biofuels have emerged as a particularly promising option due to their reduced emissions compared to fossil fuels and the availability of inexpensive feedstock, such as lignocellulosic biomass. This biomass, which is derived from plant materials, holds significant potential for biofuel production because of its abundance and sustainability. However, its chemical composition varies significantly depending on factors like plant species, age, growth stage, and seasonal variations [1].

Lignocellulosic biomass is primarily composed of cellulose (40–50%), hemicellulose (20–30%), and lignin (10–35%) [2], with trace amounts of extractives, proteins, and ash [3]. The lignin component, which provides structural support to plant cells and protects against microbial attacks, poses a challenge for biofuel production. Lignin's complex structure resists hydrolysis and fermentation, making it difficult to break down during the biofuel production process. The cellulose, which is mostly crystalline, is also resistant to enzymatic degradation, further complicating the process of converting lignocellulosic biomass into fermentable sugars [1].

To overcome these challenges, various pretreatment methods have been developed to increase the accessibility of cellulose and hemicellulose, while reducing the impact of lignin. These pretreatment methods are typically categorized into four types: physical, chemical, physicochemical, and biological pretreatments [4]. Physical pretreatment involves applying mechanical forces to the biomass to reduce its size and increase surface area, improving its accessibility for subsequent processing. Techniques such as comminution and irradiation are commonly used, but these methods typically require high energy input and yield lower amounts of fermentable sugars after hydrolysis [5, 6].

Chemical pretreatment, on the other hand, uses acids, alkalis, or ionic liquids to reduce the crystallinity of cellulose and remove lignin. While this approach can be effective in breaking down biomass, it often results in the production of inhibitory degradation products and high costs. For instance, dilute acid pretreatment can degrade fermentable sugars into harmful byproducts such as furfural and acetic acid [4], and alkali treatments may also remove some cellulose and hemicellulose, further reducing sugar yield. Additionally, chemical methods often require post-treatment washing to remove chemicals and solvents, adding to the overall process cost.

Physicochemical pretreatment, which combines physical and chemical methods, has shown better performance than either of the individual approaches. Methods such as steam explosion, ammonia fiber explosion, and supercritical carbon dioxide explosion have demonstrated success in reducing lignin content and improving the breakdown of cellulose [8, 9, 10]. However, these methods often require high energy inputs, involve harsh operating conditions, and generate significant degradation products, which limit their commercial viability.

Pulsed Electric Field (PEF) pretreatment, a form of physical pretreatment, involves applying an electric field at a specific field strength, pulse frequency, and pulse duration to biomass [11]. PEF has a distinct advantage in that it requires low energy consumption due to its rapid processing time (often as short as 100 µs per pulse) and can be performed at ambient temperatures. Unlike chemical pretreatment, PEF does not require further treatments after the process, making it more efficient and environmentally friendly. It has already been successfully applied in the medical field to treat diseases and in the food industry for various processes such as extraction and treatment of fruits, vegetables, and milk [13–16]. PEF works by disrupting the cell membranes, increasing the permeability of the cells, which can be either reversible or irreversible depending on the strength of the electric field [17, 18].

One notable application of PEF is in the biogas industry, where it has been shown to increase biogas yield from ley crop silage by 16% when applied at a field strength of 96 kV/cm with 65 pulses at a frequency of 5 Hz [19]. However, the effect of PEF on the structure of lignocellulosic biomass remains underexplored, and there is limited research on its potential to enhance the fermentation yield from lignocellulosic feedstocks.

In this context, Saskatchewan, Canada, known as the largest producer of flax in the country, offers a promising source of lignocellulosic biomass. Flax straw, a byproduct of flaxseed production, has the potential to be used as a renewable feedstock for biofuel production. This study investigates, for the first time, the applicability of PEF as a pretreatment method for flax straw. The goal is to maximize the yield of fermentable sugars and improve the efficiency of biofuel production from this lignocellulosic biomass. This research aims to contribute to the growing body of knowledge on sustainable biofuel production methods and to explore the viability of PEF as an effective, low-energy pretreatment for lignocellulosic biomass.

2. Materials and Methods

Flax straw, the lignocellulosic biomass used in this study, was sourced from a local farm in Saskatchewan. The flax straw was then milled into three distinct particle sizes for pretreatment: 0.25–0.60 mm, 0.6–0.85 mm, and 1–1.18 mm. Cellulose enzyme Cellic Ctec2, sodium acetate buffer solution, and sugar standards were purchased from Sigma-Aldrich (St. Louis, MO, USA), and were used in subsequent hydrolysis and compositional analysis. The equipment components for the Pulsed Electric Field (PEF) system were sourced from Bio-Rad Laboratories (Canada) Ltd. (Mississauga, ON, Canada), Tektronix, Inc. (Beaverton, OR, USA), Littelfuse, Inc. (Rosemont, IL, USA), and SBE Inc. (Barre, VT, USA). These components were assembled and tested to construct the PEF system.

Before building the system, simulations of the PEF unit were carried out using PSIM 11.1.7 (Powersim Inc., Rockville, MD, USA) to optimize the design parameters. The flax straw was suspended in water and loaded into the PEF chamber, which was then connected to the PEF system. The pretreatment process was conducted under varying conditions of pulse width, field strength, and number of pulses. Specifically, a screening experiment was run to evaluate the system's performance by setting the pulse width to 100 µs. The field strength was varied across three levels: 1538.5 V/cm, 4000 V/cm, and 8000 V/cm, while the number of pulses was varied between 20,000 and 120,000 pulses. The flax straw samples underwent PEF pretreatment under these conditions before being subjected to enzymatic hydrolysis.

Hydrolysis of the pretreated flax straw samples was performed using a benchtop shaking incubator (Starter Set #17002944, Bio-Rad Laboratories, Canada). The enzymatic hydrolysis process was conducted at a temperature of 48 °C, with shaking at 160 rpm, for durations of 48 and 72 hours. The pH was maintained at 5.0 by using a sodium acetate buffer solution. The enzyme loading was standardized at 0.4 mL of Cellic Ctec2 enzyme per gram of flax straw.

For the analysis of hydrolysis products, a Dionex ICS-6000 HPIC System (Thermo Fisher Scientific, Waltham, MA, USA) was used. The High-Performance Liquid Chromatography (HPLC) system was equipped with an Aminex HPX-87H column and a refractive index detector (RID). A mobile phase composed of a 5 mM aqueous solution of H_2SO_4 was used, flowing at a rate of 0.6 mL/min. The HPLC analysis was carried out at a constant temperature of 35 °C to detect and quantify glucose, cellobiose, and xylose, which were the primary sugars of interest in the hydrolysis products. The experiments were conducted in duplicate, and the average values with standard deviations were reported for each condition.

3. Results and Discussion

The pulsed electric field (PEF) setup used in this study is depicted in Figure 1, which illustrates the key components of the system: a power source, power storage capacitor, optical isolator circuit, oscilloscope, function generator/microcontroller, and high voltage probe. The system was set up with a power supply of 4 kV, and the capacitor was charged and discharged through the flax straw sample. The pulse characteristics, including pulse width and number of pulses, were controlled using the function generator or microcontroller. The maximum pretreatment conditions were set at 100 µs pulse width, 120,000 pulses, and a pulse strength of 8 kV/cm.

Following pretreatment, the flax straw was subjected to hydrolysis. The hydrolysis experiments were conducted in duplicates, and the average yield of fermentable sugars was reported along with the standard deviation. The enzymatic hydrolysis was carried out in a benchtop shaking incubator at 48 °C, 160 rpm, for 48–72 hours, with the pH maintained at 5 using a sodium acetate buffer solution. The enzyme loading was set at 0.4 mL enzyme per 1 g of flax straw.

The analysis of the hydrolysis products was performed using the Dionex ICS-6000 HPIC System, with an Aminex HPX-87H column and a refractive index detector. The mobile phase consisted of a 5 mM aqueous H_2SO_4 solution, and the flow rate was 0.6 mL/min at 35 °C. The sugars detected and quantified included glucose, cellobiose, and xylose.

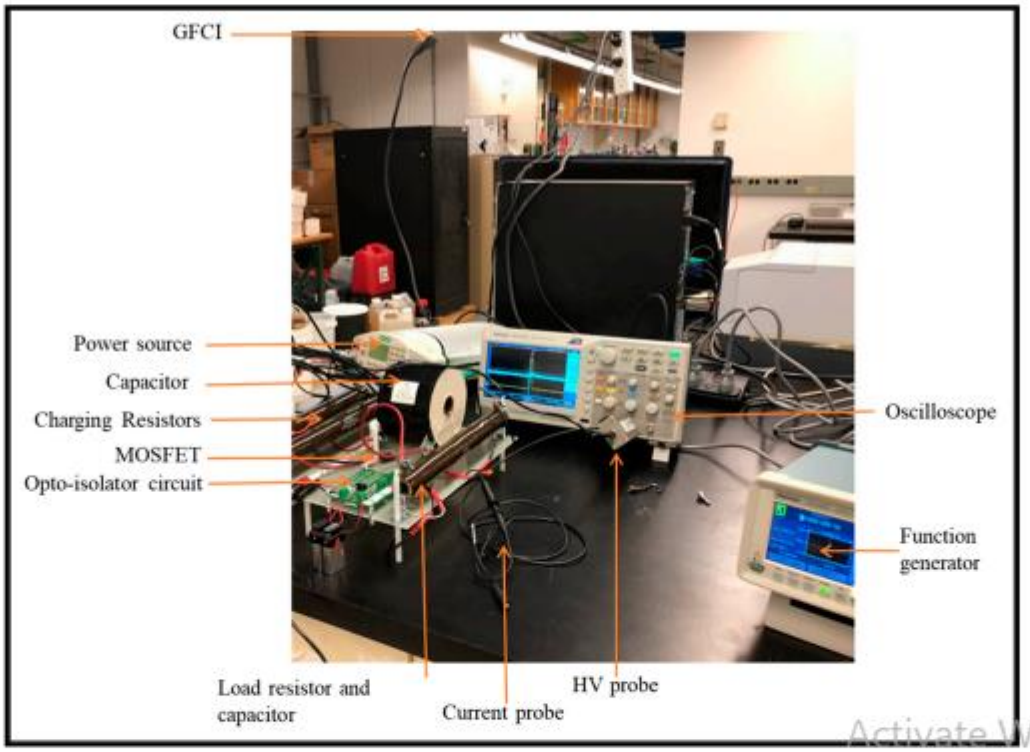

Figure 1. Pulsed electric field setup.

Effect of PEF on Flax Straw (Particle Size 1–1.18 mm)

Table 1 shows the results of the hydrolysis of pretreated flax straw with a particle size of 1–1.18 mm. The flax straw was pretreated using PEF at 1538.5 V/cm, 100 µs pulse width, and 40,000 pulses. After 48 hours of hydrolysis, there was no significant change in the yields of cellobiose, glucose, or xylose, as seen in Table 1. It is noted that using a larger particle size for pretreatment helped to reduce the energy consumption associated with milling the straw prior to the PEF treatment.

Component	Yield (%) with Respect to Unpretreated Straw
Cellobiose	-1 ± 8
Glucose	-1 ± 2
Xylose	-7 ± 14

Effect of PEF on Flax Straw (Particle Size 0.25–0.60 mm)

Table 2 presents the results from hydrolysis of pretreated flax straw with a particle size of 0.25–0.60 mm, which was pretreated at 4000 V/cm, 100 µs pulse width, and 80,000 pulses. Despite an increase in the number of pulses, the relative change in sugar yields remained insignificant even after 72 hours of hydrolysis. This suggests that the lower energy and pulse settings did not sufficiently alter the structure of the biomass to enhance the hydrolysis process.

Component	Yield (%) with Respect to Unpretreated Straw
Cellobiose	-36 ± 8
Glucose	-1 ± 8
Xylose	3 ± 3

Effect of PEF on Flax Straw (Particle Size 0.60–0.85 mm)

Table 3 shows the hydrolysis results for flax straw with particle sizes of 0.60–0.85 mm. The flax straw was pretreated using PEF at varying conditions, with field strengths of 1538.5 V/cm, 4000 V/cm, and 8000 V/cm, pulse widths of 100 µs, and pulse numbers of 20,000, 40,000, 60,000, and 120,000. The hydrolysis was conducted for 48–72 hours. The results show that the yield of fermentable sugars (cellobiose, glucose, and xylose) increased significantly as the field strength, number of pulses, and hydrolysis time increased.

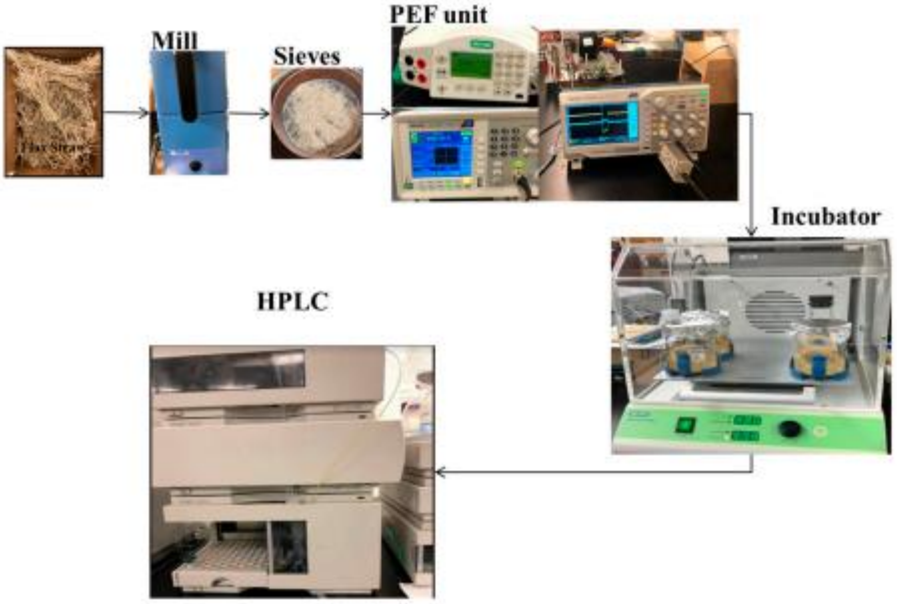

Figure 2. Setup of PEF unit and hydrolysis.

The maximum yield of fermentable sugars was obtained at 8000 V/cm, 120,000 pulses, and 72 hours of hydrolysis, with relative yields of 67% for cellobiose, 25% for glucose, and 12% for xylose. This condition demonstrated the highest sugar yield, and the differences between 60,000 and 120,000 pulses were minimal, suggesting that 60,000 pulses could be considered the optimum setting for energy efficiency.

Field Strength (V/cm)	Pulse Width (µs)	Number of Pulses	Hydrolysis Time (h)	Cellobiose (%)	Glucose (%)	Xylose (%)
1538.5	100	20,000	48	15 ± 5	20 ± 2	52 ± 3
1538.5	100	40,000	48	27 ± 11	13 ± 0	66 ± 3
4000	100	80,000	72	28 ± 7	-7 ± 2	-4 ± 2
8000	100	60,000	72	59 ± 19	24 ± 11	11 ± 6
8000	100	120,000	72	67 ± 1	25 ± 2	12 ± 0

The data from the hydrolysis of PEF-pretreated flax straw clearly shows that PEF treatment enhances the yield of fermentable sugars. The maximum yield was achieved at the highest field strength (8000 V/cm) and the highest number of pulses (120,000), with a 72-hour hydrolysis time. The yield increases were most notable for cellobiose (67%), glucose (25%), and xylose (12%) compared to unpretreated flax straw. This suggests that PEF pretreatment effectively disrupts the structure of flax straw, making it more accessible for enzymatic hydrolysis.

Further investigations are recommended to screen a wider range of pretreatment conditions and perform detailed structural analyses of the pretreated flax straw using techniques like scanning electron microscopy (SEM), X-ray diffraction (XRD), Fourier-transform infrared spectroscopy (FTIR), and Brunauer–Emmett–Teller (BET) surface area analysis. These analyses will help clarify the mechanisms behind the observed yield improvements following PEF pretreatment.

4. Conclusions

The study successfully designed and tested a Pulsed Electric Field (PEF) system for the pretreatment of flax straw, with the aim of enhancing the yields of fermentable sugars during subsequent hydrolysis. The PEF system's operational parameters were optimized to a pulse width range of 75–100 µs, a field strength range from 192 to 8000 V/cm, and a number of pulses between 20,000 and 120,000. For the pretreatment of flax straw, the system was operated at a pulse width of 100 µs and a field strength of 4 kV/cm, with the number of pulses varying from 20,000 to 120,000.

The effectiveness of the PEF pretreatment was assessed based on flax straw particle sizes of 0.25–0.60 mm, 0.6–0.85 mm, and 1–1.18 mm. The results showed that flax straw particles sized at 0.25–0.60 mm and 1–1.18 mm did not exhibit significant improvements in the yields of fermentable sugars, such as glucose, cellobiose, and xylose, after the PEF pretreatment. In contrast, flax straw with a particle size of 0.6–0.85 mm showed notable improvements in sugar yields after treatment with PEF. Specifically, the maximum relative yields of glucose, cellobiose, and xylose increased by 25%, 67%, and 12%, respectively, compared to the untreated flax straw, when subjected to 120,000 pulses at a field strength of 8000 V/cm.

These findings suggest that PEF pretreatment can significantly enhance the conversion efficiency of flax straw to fermentable sugars, particularly when the particle size is within the 0.6–0.85 mm range. However, the results also indicate that the effectiveness of the PEF treatment is dependent on the particle size of the flax straw, with certain particle sizes not showing significant improvements in sugar yields.

Further investigation is required to better understand the impact of PEF on the structural changes in flax straw during pretreatment. This would involve detailed analysis of the flax straw’s chemical and physical structure post-PEF treatment to identify any changes in lignin, cellulose crystallinity, and other components that might explain the observed variations in sugar yield improvements. Additionally, optimizing the PEF parameters such as pulse width, field strength, and number of pulses, while considering other potential pre-treatment methods, could further enhance the overall yield and efficiency of biofuel production from lignocellulosic biomass.

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