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Abstract

In recent years, the second-generation bioethanol and advanced bio-based material production from biomass are focused on the pretreatment process by separating cellulose components from other components such as lignin and hemicellulose. Therefore, a physicochemical pretreatment method is needed by applying non-thermal pulsed electric field (PEF) and alkali methods to increase the cellulose availabilities with a short process and low energy input. The aim of this study was to analyze the lignocellulose content of corncob biomass by using non-thermal pulsed electric fields (PEF) and NaOH pretreatment. The pretreatment factors used were the electric field strength of PEF and the pretreatment time. Analysis of the structure and elements of the lignocellulose based on the characteristics of the gravimetric method and SEM-EDX for control and treated samples. The results showed that pretreatment of corncobs biomass by using PEF optimally at an electric field strength of 9 kV/cm and pretreatment time of 60 seconds that was increasing cellulose of 40.59% when compared with the control and also decreasing the hemicellulose and lignin content of 12.9% and 2.02%, respectively. Under these conditions, the energy per pulse and specific input energy of PEF required 0.0205 J and 8.72 kJ/L, respectively. The microstructure analysis by using SEM-EDX showed significantly visual differences and was an increase in the percentage of C and O atoms between untreated and treated corncob biomass. Furthermore, the corncob biomass treated by using non-thermal PEF and alkali can effective and efficient for the next process into cellulose-derived products.

Keywords: *corncob biomass; pulsed electric field; NaOH; pretreatment; cellulose*

INTRODUCTION

The use of biomass as the main resource for the production of second-generation bioethanol and subsequent conversion processes into advanced biomaterials has become the main focus of several countries in the world. Lignocellulose as an alternative raw material for bioethanol has advantages such as low energy, abundant availability, low cost, and higher bioethanol yield. Thus, the utilization of biomass waste has been projected in sustainable development to help reduce deforestation by reducing our dependence on forest woody biomass to produce biofuels (Saini *et al*., 2015). The problem that arises in the process of converting biomass feedstock into biofuel lies in the cell wall of biomass which has an integral structural complexity of the lignocellulose fraction and as a strong barrier of inhibitors and by-products produced during pretreatment (Kumar & Sharma, 2017).

Therefore lignocellulosic binding structure consisting of cellulose, hemicellulose, and lignin must be broken down. Also the lignin content must be removed through the pretreatment process. In general, the stages in the biomass pretreatment process include the process of damaging hydrogen bonds in crystalline cellulose, then the process of breaking the matrix of hemicellulose and lignin, and the final process is increasing porosity and surface area of cellulose for subsequent enzymatic hydrolysis (Li *et al*., 2010). Some criteria to be considered in choosing a pretreatment method include low energy costs, the ilvolvement of pretreatment catalysts with low processing costs, efficient processing time which can later have an impact on the downstream process stages and commercialization related to operating costs, capital costs and biomass costs (Kumar & Sharma, 2017). Therefore, the application of appropriate pretreatment methods for the deconstruction of cell wall structures during the conversion process is also a matter of concern.

The application of non-thermal Pulsed Electric Field (PEF) technology with low electric field strengths between 5-20 kV/cm with a short time can damage the cellulose bonds to other sugar groups/elements and cause the separation of lignocellulose bonds (Kumar & Sharma, 2017). The advantage of using PEF is that it is very short on time, low power requirements and simple instrument design (Kumar *et al*., 2009). However, the use of PEF in the pretreatment process must also consider the electric field strength and pretreatment time since it is closely related to the energy needed by the PEF during the pretreatment process. The aim of this study was to analyze the lignocellulose content of corncob biomass by using non-thermal PEF and alkali pretreatment. Corncob biomass waste was chosen not only because of its sub-optimal utilization but also has a high cellulose content compared to other biomass wastes (Kumar *et al*., 2009). It is expected that the non-thermal pretreatment process using PEF and NaOH can effectively and efficiently reduce lignin and hemicellulose and increase cellulose content in corncob biomass. Hence it can support the production of second-generation bioethanol and subsequent conversion processes into several other advanced biomaterial products.

MATERIALS AND METHOD

Apparatus and Materials

The main apparatus used in this study is a laboratory-scale PEF and the installation scheme

represented in Figure 1. The treatment chamber of PEF has a maximum capacity of 13 L made from stainless steel which is safe and resistant to alkaline or acidic chemicals. Negative and positive electrodes installed in the treatment chamber are also made of stainless steel with a distance between the electrodes of 3.25 cm. The PEF generator contains several electronic circuits to produce high-voltage electrical pulses. The control panel provides the power button, speed control button, input voltage regulator, high voltage button, timer (OMRON type H5CX-AN) and input voltage display. The PEF design was modified and adapted to the needs of the corncob biomass pretreatment process for laboratory scale.

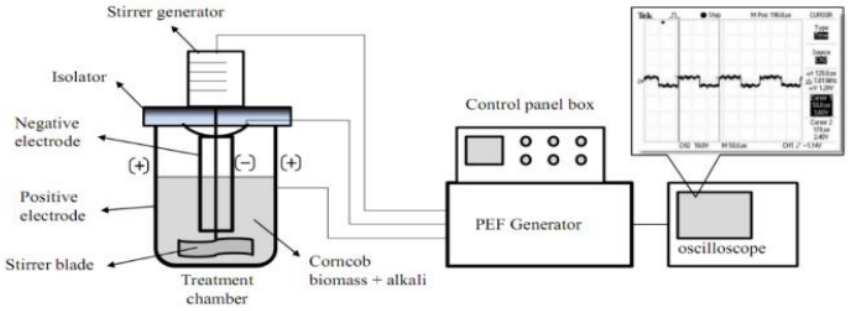

Figure 1. Pulsed electric field installation scheme

In addition, the PEF apparatus also has been calibrated between the input voltage and the output voltage as shown in Figure 2, while the measurement of frequency and pulse width also measurements using an oscilloscope.The PEF apparatus has an output voltage specification between 8-31.36 kV, the electric field strength (Ef) produced 2.46-9.65 kV/cm, the input frequency of 7.813 kHz, the square pulse generated has a pulse width of 66 µs, and the stirrer speed of 50-200 rpm. While the material used in this study was corncobs as biomass waste obtained from farmers in Malang-Indonesia, distilled water, NaOH, and H2SO4 pa (Merck).

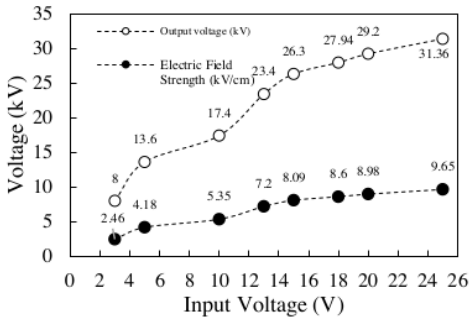

Figure 2. The voltage calibration in PEF apparatus

Corncob Pretreatment

Dried corncobs milled and sieved 60 mesh, then added 60% NaOH solution with a ratio of 1:10 (w/v) and filled into the treatment chamber. The input voltage of PEF is set to produce electric field strength at 5 and 9 kV/cm andpretreatment time is set at 60, 180 and 300 seconds. The stirring process (100 rpm) is carried out during the pretreatment process. Furthermore, corncobs powder mixed with NaOH from the treatment chamber then filtered and neutralized with distilled water

Analysis Method

The analysis of cellulose, hemicellulose, and lignin was conducted by using the Chesson method (Datta, 1981). While, the energy needs analysis in this study is based on the energy value per pulse and specific input energy according to the equation below: Calculation of energy per pulse (J):

$$W_{\text{pulse}} = U \; x \; I \; x \; \tau \qquad (1)$$

Calculation of specific input energy (kJ/L) with the equation:

$$W_{spec} = \frac{f(t)}{Vol} \; x \; W_{pulse} \; \tau \qquad (2)$$

Where Wpulse is the energy per pulse (J), U is the PEF voltage (V), I is the current (A) and τ is the pulse width (s). While Wspec is the specific input energy (kJ/L), f is the frequency (Hz), t is the pretreatment time and Vol is the volume of material inside the treatment chamber (L). The microstructure analysis was carried out with the principle of visual image detection through scanning electron microscopy (FEI Inspect S50 Genesis). Microstructure analysis using SEM is equipped with EDX-EDAX analysis which is used to evaluate morphological changes and elements analysis of untreated and treated samples.

Table 1. The lignocellulose content of corncob biomass on the variation of electric field strength and pretreatment time

Pretreatment variables		Lignocellulose content		
Ef (kV/cm)	Pretreatment time (second)	Cellulose (%)	Hemicellulose (%)	Lignin (%)
Untreated samples		22.50	38.32	11.72
	60	59.84±0.11	28.58±1.48	9.61±0.54
5	180	56.63±2.48	30.17±2.81	9.54±0.09
	300	61.21±0.43	28.41±0.29	9.80±0.12
	60	63.09±1.73	25.42±0.66	9.69±1.21
9	180	62.76±0.08	25.91±0.22	9.32±0.16
	300	62.64±0.53	26.03±0.39	9.97±0.09

RESULTS AND DISCUSSION

Lignocellulose Content

Corncob biomass without treatment and treated by varying the electric field strength and pretreatment time, containing cellulose, hemicellulose, and lignin, as indicated in Table 1. The highest component of corncob biomass (untreated) is hemicellulose (38.32%), and followed bycellulose content (22.5%) and lignin content (11.72%). Based on Table 1, it can be seen that the cellulose content of corncob after pretreatment has increased cellulose with a range between 56.63±2.48% - 63.09±1.73%. The optimum increase incellulose content (63.09±1.73%) carried out at the electric field strength of 9 kV/cm and pretreatment time of 60 seconds.The increased pretreatment time (up to 300 seconds) on the electric field strength of 9 kV/cm, did not show a significant increase in cellulose and decrease in hemicellulose. However, the difference in the strength of the electric field provided can increase cellulose and at the same time decrease hemicellulose of corncob biomass which is quite significant. Therefore, the electric field strength factor has a major role in the PEF pretreatment process to damage lignocellulosic bonds.

A significant increase in cellulose content between untreated and treated samples proved that the pretreatment method using non-thermal PEF and NaOH was able to increase the cellulose content of corncob biomass. The electric field strength that is exposed to the surface of corncob biomass aims to damage the structure of hydrogen bonds that connects lignin-hemicellulose and lignin-cellulose so that the lignocellulosic bonding structure undergoes irreversible termination. In such conditions, NaOH added during the pretreatment process will easily dissolve lignin and hemicellulose and other amorphous particles. The phenomenon of breaking hydrogen bonds together with alkali dissolution processes can also change the cellulose crystal structure and produce better cellulose chains (Suryanto *et al*., 2017).

In addition, the reduced content of lignin, hemicellulose, and other particles will increase the degree of crystallinity of cellulose. The crystallinity index also increases with increasing crystal size because the surface of the crystal corresponds to the reduction of amorphous particles that protect cellulose (Kim *et al*., 2010). On the other hand,the higher crystallinity index of cellulose also has great potential to produce micro crystalline cellulose (MCC) or even become nano crystalline cellulose (NCC), bioplastics and other derivative products.

Hemicellulose is a compound that makes up plant cell walls together with cellulose and lignin. Table 1 shows the hemicellulose content of corncob biomass has decreased 12.9% when compared with untreated samples. The highest decrease in hemicellulose content occurred in the variation of electric field strength treatment 9 kV/cm and pretreatment time of 60 seconds. It is also the same as the PEF treatment to produce the highest increase in cellulose. Therefore, there is a correlation between reducing hemicellulose content and increasing cellulose content. Hemicellulose has characteristics that are relatively sensitive to operating conditions. Although in this study the variation of pretreatment time did not have a significant effect, but the pretreatment time had to be controlled to avoid the formation of undesirable products such as furfurals and hydroxymethyl furfurals which could later inhibit the subsequent downstream process,like the fermentation process (Mood *et al*., 2013).

Lignin is a component that protects cellulose in plant cell walls. One of the main objectives in the biomass pretreatment process is delignification or reduction in lignin content which will be accompanied by an increase in cellulose in corncob biomass. In addition, another purpose of delignification is to facilitate the enzymatic saccharification process which is an important parameter in the pretreatment process (Li *et al*., 2010). Table 1 shows the lignin content decreased between 1.75% - 2.4% when compared with untreated sample. The highest reduction in lignin content (9.32±0.16%) occurred in the treatment of

electric field strength of 9 kV/cm and pretreatment time of 180 seconds.

The physical pretreatment method has a minor effect in reducing lignin (Mood *et al*., 2013), therefore an sodium hydroxide solvent was added to dissolve the lignin structure when high voltage pulses have damaged the hydrogen bond with hemicellulose. The alkalinisation pretreatment process using NaOH has the advantage that it does not require a complex reactor so that it is easy to apply and can be carried out at room temperature (Mood *et al*., 2013). On the other hand, the use of alkaline or acidic solvents in conventional chemical pretreatment still has limitations such as being corrosive, toxic and not in line with the principles of green technology indeed. Therefore it is recommended for the biomass pretreatment process using green solvents that require low pressure, stable at room temperature and non-flammable such as deep eutectic solvent (DES) (Tan *et al*., 2019).

Energy Analysis During Pretreatment

Analysis of energy requirements during the pretreatment process with PEF is an important parameter in maintaining the characteristics of non-thermal treatment. The energy needed during the treatment process using PEF consists of the calculation of energy per-pulse and specific input energy. Energy per- pulse is the amount of energy given to the series per- pulse magnitude expressed in Joules. The specific input energy is the energy given for each unit volume of material during the pretreatment process with PEF expressed in units of kJ/L. Based on equations (1) and (2), the energy per-pulse and specific input energy can be calculated which can be seen in Table 2.

Table 2. The specific input energy of PEF during the pretreatment process

Electric Field Strength (kV/cm)	Pretreatment time (second)	Energy per Pulse (Joule)	Specific Energy Input (kJ/L)
5	60	0.0114	4.84
5	180	0.0114	14.53
5	300	0.0114	24.22
9	60	0.0205	8.72
9	180	0.0205	26.16
9	300	0.0205	43.60

The energy per pulse needed by PEF is positively correlated with the output voltage generated, current and pulse width. Since the current value used and the pulse width produced by the PEF apparatus are the same, the amount of energy per pulse is determined by the output voltage and the electric field strength of PEF. The higher the value of the electric field strength, the greater the energy per pulse needed by PEF to generate high voltage pulses. However, a high energy per pulse value does not always cause a high mass transfer, but it is also adjusted to the condition of the cell to be treated by PEF. The total permeabilization of plant cell tissue is obtained by applying either a very high pulse energy or several low energy per pulses (Donsi *et al*., 2010). Based on Table 2 it can also be seen that the specific input energy needed during the pretreatment process of corncob biomass with PEF is between 4.84 - 43.60 kJ/L.

In this study, the highest specific input energy value (43.60 kJ/L) is still relatively low for energy requirements during the biomass pretreatment. When converted to electrical power used, the highest specific input energy PEF is 0.73 kWh, while in the best treatment, the electrical energy needed is 0.15 kWh. This is also supported by the application of low electric field strength (9 kV/cm). The higher electric field strength application (above 35 kV/cm) is suitable for bacterial inactivation process, while the application of low electric field strength (1-10 kV/cm) is suitable for increasing mass transfer in the extraction of important antioxidant compounds (carotenoids, phenolics, and anthocyanins) from agricultural materials and biomass pretreatment processes (Donsi *et al*., 2010; Putranto *et al*., 2014; Izza *et al*., 2016; Putranto *et al*., 2018; Dewi *et al*., 2019). The low specific input energy will certainly also affect cheaper production costs, so the pretreatment process with PEF has the potential to be applied on an industrial scale.

Microstructure and Element Composition Analysis

The morphological structure of corncob biomass was observed using SEM-EDX to evaluate changes in external structure caused by pretreatment treatment. Corncob biomass with an electric field strength treatment of 9 kV/cm and 60 seconds pretreatment time and untreated samples then performed microstructure and element composition testing with SEM-EDX. The electric field strength from PEF have the effect of forming gaps and preferential pathways in the membrane cell structure. This is also evidenced from the SEM results between the untreated and treated of corncob biomass at 100, 250 and 500 magnification (Figure 3).

The morphological structure in untreated corncob biomass at various magnifications has a clear visual differences when it was compared with treated by PEF-NaOH. The structure of corncob biomass has amorphous-looking fibers, many flakes of several types of fiber, and many celluloses which are still in the form of bundles. While, the microstructure of the sample after the pretreatment was seen one type of fiber bundle that could split into single cellulose fiber. This also shows that the non-thermal treatment method of PEF-NaOH can damage the lignocellulosic structure of the biomass and at the same time dissolve amorphous fiber portions so that one component and bundle fibers appear.

The NaOH solution can penetrate the intermediate part of the crystallite and destroy the inter and intra-hydrogen bonds between the cellulose molecule and the surrounding crystalline area so that the amorphous fraction can be more hydrolyzed and the total amount of amorphous cellulose can be reduced. This is because NaOH dissociates in the solvent fraction to Na + and OH- ions. The OH- ion will attack C from the C = O bond in lignin and will result in the formation of a tetrahedral shape but can change immediately when the alkoxide leaves the

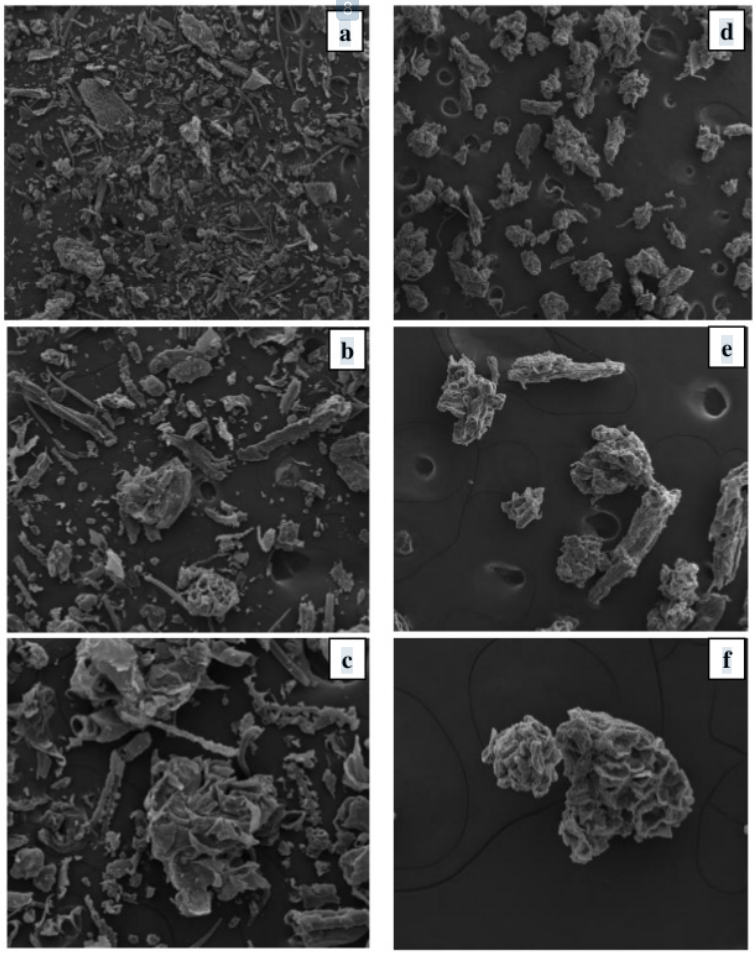

Figure 3. SEM results of untreated corncob biomass with magnifications of 100 times (a), 250 times (b) 500 times (c) and treated corncob biomass with magnifications of 100 times (d), 250 times (e) 500 times (f)

carboxylic acid. In very fast reactions, alkoxides act as bases and deprotonate carboxylic acids.

The application of high voltage pulses PEF leads to the induction of critical electric potential across the cell membrane, thus causing local structural changes and disruption of cell wall membrane integrity. Increased mass permeability causes pores or cracks that damage the plant's main cell wall. Since plant cell membranes consist of cellulose, hemicellulose, and lignin, the pores that occurs in the primary cell wall due to PEF indirectly also damages the bonds between lignocellulose in biomass. Under these conditions, the NaOH solution that is in the treatment chamber can easily get into the macrofibril fiber to carry out the delignification process. In addition, degradation of amorphous cellulose fraction also becomes easier and requires a shorter time. In this study, a quantitative analysis of changes in microstructure between untreated and treated corncob biomass by using SEM-EDX represented in Figure 4.

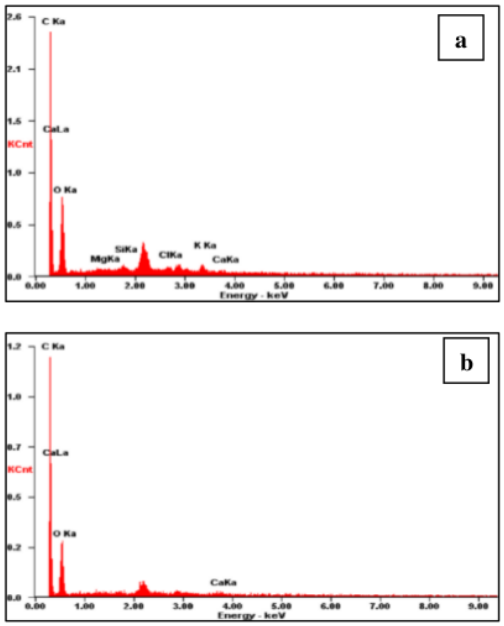

Figure 4. SEM-EDX microanalysis of untreated (a) and treated corncob biomass (b)

Figure 4a show atoms detected in the untreated corncob biomass such as atoms C, O, Cl, K, Mg, Si and Ca. Whereas in Figure 4b, only 3 atoms were detected in the trated corncob biomass, namely C, O and Ca. This shows that the existence of PEF (electric field strength of 9 kV/cm, pretreatment time of 60 second) and sodium hydroxide solvent can reduce some hemicellulose and lignin compounds and other amorphous compounds characterized by the reduction of atoms from 7 atoms to 3 atoms. Quantitative data on the percentage of each atom of the control sample and the PEF-NaOH treatment sample as well as a comparison with another study is shown in Table 3.

Table 3. Percentage of atoms detected using SEM EDX microanalysis

Atoms	Raw Corncob Biomass (untreated)		Treated Corncob Biomass	
	Weight (%)	Amount (%)	Weight (%)	Amount (%)
Present study:				
C	51.67	59.91	56.52	63.66
O	43.98	38.29	42.63	36.05
Mg	0.54	0.31	-	-
Si	0.76	0.37	-	-
Cl	1.00	0.39	-	-
K	1.58	0.56	-	-
Ca	0.48	0.17	0.86	0.29
Ayeni & Daramola, (2017) study:				
C	47.9	56.5	49.17	56.38
O	42.47	35.88	46.13	39.71
Mg	0.38	0.22	0.42	0.24
Si	0.21	0.11	0.34	0.17
Cl	-	-	-	-
K	5.75	2.08	0.07	0.02
Ca	0.13	0.08	0.39	0.13

Based on Table 3, the raw corncob biomass (untreated) has 7 types of atoms detected, whereas in the treated corncob biomass (electric field strength of 9 kV/cm and pretreatment time of 60 second) there are only 3 types of atoms detected namely C, O, and Ca. The atomic weight of C in the treatment sample increased from 51.67% to 56.52% when compared to untreated corncob biomass. While the percentage of the number of C atoms in the treated corncob biomass also increased from 59.91% to 63.66%. The weight of Ca atoms also increased from 0.48% to 0.86%, with the percentage of the number of Ca atoms also increasing from 0.17% to 0.29%. The presence of several other atoms (Mg, Si, Cl, and K) was not detected. It indicates that there are has no fibers contained in the treated corncob biomass.

Another studies by Ayeni & Daramola, (2017), with the thermal pretreatment (120˚C, pretreatment time of 30 minutes) and adding 1% (v/v) hydrogen peroxide solution and 0.1g NaOH/g biomass, as the highest value of cellulose yield (58,66%), showed a higher percentage of atomic O values and lower of C and Ca atoms compared with the present study. The higher percentage values of O atom was probably the action of hydrogen peroxide which more pronounced during pretreatment as revealed in the cellulose content retained (Ayeni & Daramola, 2017). On the other hand, the thermal pretreatment by adding of sodium hydroxide and hydrogen peroxide solution still has some fibers or other inorganic elements which are indicated by the presence of detected atoms such as Mg, Si, and K even in low numbers.

The presence of several fibers and other elements besides the constituent cellulose is also related to the amount of cellulose content in the treated corncob biomass. In this study, the high percentage of

C atom value (63.66%) and the absence of other atoms making up cellulose, showed a high cellulose content of treated corncob biomass (63%). This is also in line with Ayeni & Daramola, (2017) study, where the highest percentage of C atoms (56.38%) also shows the highest cellulose content (59%) of treated corncob biomass. However, the non-thermal pretreatment results in higher cellulose and C atom content compared with thermal pretreatment. Therefore, it can be said that the non-thermal pretreatment by using PEF-NaOH could be effective and efficient to reduce lignin and hemicellulose and increase cellulose compounds in corncob biomass.

The C, O and H atoms are the atoms in the cellulose monomer aldehyde group. However, in this study H atoms cannot be detected by SEM-EDX due to the very small number of H atoms or 1 electron. Therefore further research is recommended to use X-Ray Diffraction (XRD) analysis to determine the presence of C, O and especially H atoms. However, even though the H atom is not detected at all in microanalysis using SEM-EDX, the C and O atoms increasing both the amount and the weight are the main indicators that the compounds detected in SEM-EDX in the treated sample are cellulose compounds.

CONCLUSION

The gravimetric analysis and SEM-EDX microanalysis showed that the non-thermal PEF pretreatment method can effectively and efficiently increase the cellulose content of corncob biomass. An electric field strength of 9 kV/cm and the pretreatment time for 60 seconds shows the highest increase in cellulose content, decreased hemicellulose and lignin content. Under these conditions, low energy is needed during non-thermal PEF-NaOH pretreatment. The electric field strength of PEF has a significant influence on the cellulose content of corncob biomass pretreatment. The microstructure analysis by using SEM-EDX showed significantly visual differences and was an increase in the percentage of C and O atoms between untreated and treated samples. Furthermore, the corncob biomass treated by using non-thermal PEF and alkali can effective and efficient for the next downstream process as converted into commercial cellulose-derived products.

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