

A study of the porosity of coconut dregs cells based on variations in substrate size and pulsed electric field pre-treatment

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Abstract

This study aims to analyze the porosity of coconut dregs cells based on observations using FE-SEM, resulting from variations in substrate size and the duration of pulsed electric field (PEF) exposure, as well as delignification by *Agrobacterium* sp. The study was conducted using an exploratory approach, and the data were analyzed using Origin Lab. software. PEF is a non-thermal physical treatment with relatively low energy and short duration. The treatments in this study involved variations in coconut dregs particle size (20, 40, and 60 mesh) and variations in the contact time between the coconut dregs and PEF (30, 60, and 90 seconds). PEF was applied at 7900 V/cm, a frequency of 9 kHz, and a distance of 10 cm between the anode and cathode. Cell porosity was observed to determine the effects of the initial PEF treatment and degradation by *Agrobacterium* sp. Findings tested at a significance level of 0.001 indicated that the duration of PEF contact significantly influenced cell porosity as observed by FE-SEM. Longer PEF contact resulted in deeper pores, which correlated with the size of the coconut dregs particles.

Keywords: *Agrobacterium* sp.; Coconut dregs; FE-SEM; PEF; Porosity

1. Introduction

Biomass fractionation has recently become a rapidly growing field of research, and at the same time, it presents a significant challenge in terms of implementing appropriate technologies. For example, the disruption of cell membranes during biomass separation is a crucial step in the process (1). Efforts to disrupt the biological structure of a material are undertaken to exploit the trapped components (2,3). These include physical, chemical, biological, physicochemical, and combined processes (3). The application of pulsed electric field (PEF) fractionation technology utilizes high-voltage electric fields and short pulses (1). PEF is a non-thermal physical pretreatment that utilizes electric fields, requiring neither high energy nor high costs (2). PEF is applied by briefly passing the sample between two high-voltage electrodes (4). PEF is widely applied in the development of environmentally friendly and sustainable extraction or bio-suspension techniques for plant materials (5).

Electroporation of biological cell membranes for the processing of a lignocellulosic material following preliminary PEF treatment results in increased membrane permeability to molecules that are normally impermeable (6). PEF applied at a frequency of 1 Hz, an amplitude of 500 V cm⁻¹, and a duration of 1 minute induces electroporation of the constituent endothelial cells, thereby opening transcellular pathways. Meanwhile, PEF applied for >30 minutes at a frequency of 200 Hz and an amplitude of 25 V/cm can break bonds and increase permeability through paracellular pathways (7). The application of a moderate-strength electric field for a short duration between 5 and 20 kV/cm can damage lignocellulosic structures (8). Cell membranes undergo local structural changes and electrical damage when a critical

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electric potential is reached. This increases membrane permeability and facilitates the entry of intracellular chemicals into the surrounding solution (6).

In this study, the initial PEF treatment was used to break down the lignocellulosic cell walls. Meanwhile, the biological treatment involved delignification by *Agrobacterium* sp. using low energy and without chemical inputs. *Agrobacterium* sp. produces specific enzymes that facilitate lignin degradation, such as DyP-type peroxidase and β -etherase (9), and this process can occur more rapidly than with fungi. Pre-treatment can accelerate fiber dissociation, enhance biomass conversion, and hydrolyze polysaccharides into monosaccharides. Pre-treatment also reduces lignin content, cellulose crystallinity, and energy consumption. Pre-treatment is a crucial step for altering the structure of stubborn lignocellulose, partially hydrolyzing acid-soluble lignin, and disrupting the crystalline structure of cellulose. Furthermore, pre-treatment can accelerate subsequent processes to be applied (10).

Sources of lignocellulose are widely available, primarily in the form of agricultural waste. One type of agricultural waste containing lignocellulose that has not been widely utilized is coconut husk. Coconut dregs, a byproduct of coconut milk extraction, is known to retain nutrients and also contain lignocellulose. The city of Padang produces 1.2 tons of coconut dregs annually (11). West Sumatra is indeed known as a region with a high consumption of coconut milk. Consequently, coconut milk extraction businesses are common in traditional markets and residential areas. Therefore, this study aims to analyze the porosity and cell surface of coconut dregs following preliminary PEF treatment followed by delignification using *Agrobacterium* sp.

2. Methodology

2.1. Materials

The main materials used in this study were coconut dregs, pure cultures of *Agrobacterium* sp., LB medium, and homemade M9 minimal medium.

2.2. Equipment

The main equipment required for this study included a PEF, an FEI Inspect F50 FE-SEM with a 12.5 mm WD lens, and a Unimax 1010 shaker incubator.

2.3. Methods

The experimental design used in this study was exploratory, consisting of three treatments: coconut dregs particle size (20, 40, and 60 mesh), contact time (30, 60, and 90 seconds), and incubation time with *Agrobacterium* sp. (5, 7, and 9 days). Observations were made on the porosity and cell surface of the coconut dregs based on FESEM data.

2.4. Research Implementation

2.4.1. Sample Preparation

Coconut dregs were obtained from coconut milk pressing operations at a traditional market in Padang City. The coconut dregs were dried using a cabinet dryer until the moisture content reached a maximum of 14%. The coconut dregs were then ground to sizes of 20, 40, and 60 mesh.

2.4.2. PEF Setup

The main equipment used in this study was the PEF (PEF Setup, Figure 1). The PEF treatment chamber was made of acrylic; the negative and positive electrodes were installed in the treatment chamber with a distance of 10 cm between them. The PEF generator contained several electronic circuits to generate high-voltage electrical pulses. The control panel features a power button, a speed control button, an input voltage regulator, a high-voltage button, a timer (OMRON type H5CX-AN), and an input voltage display.



Figure 1 PEF Setup

2.4.3. Initial Treatment with PEF

The PEF voltage was set to produce an electric field strength of 7900 V/cm, a frequency of 9 kHz, and a pulse width of 27 μ s; the initial treatment duration was divided into 30, 60, and 90 seconds.

2.4.4. *Agrobacterium* sp. Inoculum Preparation (12)

Agrobacterium sp. cultures obtained from the Bioindustry Laboratory, Faculty of Agricultural Technology, Brawijaya University, were revived on LB medium at 30°C for 24 hours and stored at 4°C. The cells were then harvested by centrifugation (5000 rpm, 10 minutes), and the cell pellets were washed with minimal M9 medium, then washed again by repeated centrifugation and resuspended in minimal M9 medium.

2.4.5. Biodegradation (12,13)

A 4-mL suspension of *Agrobacterium* sp. was inoculated into 400 mL of minimal M9 medium containing 40 g of sterile coconut dregs (according to treatment), pH 6.5, and incubated in a shaking incubator at 180 rpm for 3 days at 37°C. At the end of the process, the degraded samples were filtered, washed with distilled water, and dried. Observations were made of the surface of the coconut dregs cells.

2.5. FE-SEM Observations (14)

Morphological analysis was performed using an FEI Inspect F50 FE-SEM with a 12.5 mm WD lens and 500x magnification.

2.6. Porosity (15)

The FE-SEM images were processed using Origin Lab. Software. Import the SEM image into the Origin Lab. Software interface, click the Metrics toolbar, and metric data values will appear. Convert the metric data into a 2D graph by clicking the Plot toolbar, which will yield the Hmax and Hmin values. Click Metrics > Dimensions > Table to determine the X and Y values. Click Metrics > Analysis > Mathematics > 2D to obtain the integral volume value. Total volume is obtained from the value $X*Y*Z$, where the Z value is derived from the difference between Hmax and Hmin. Solid volume is obtained from the difference between the integral volume and the area under the curve; the area under the curve is the result of $X*Y*Hmin$. Calculate the pore volume from the difference between the total volume and the solid volume. Porosity is calculated using the following equation:

$$\text{Porosity (\%)} = \frac{\text{Pore area}}{\text{Total area}} \times 100 \quad (1)$$

3. Discussion

The FE-SEM image shows the effect of PEF on coconut dregs cells, specifically its impact on cell structure. (16) explains that FE-SEM images are very useful for obtaining observations regarding porosity. Figure 2, which illustrates the changes in porosity of coconut dregs cells treated with varying PEF exposure times and coconut dregs particle sizes, shows that PEF exposure time significantly affects porosity. A PEF contact time of 90 seconds and a coconut dregs size of 20 mesh yielded a porosity of 45.96%, a coconut dregs size of 40 mesh yielded a porosity of 42.81%, and a coconut dregs size of 60 mesh yielded a porosity of 47.44%.

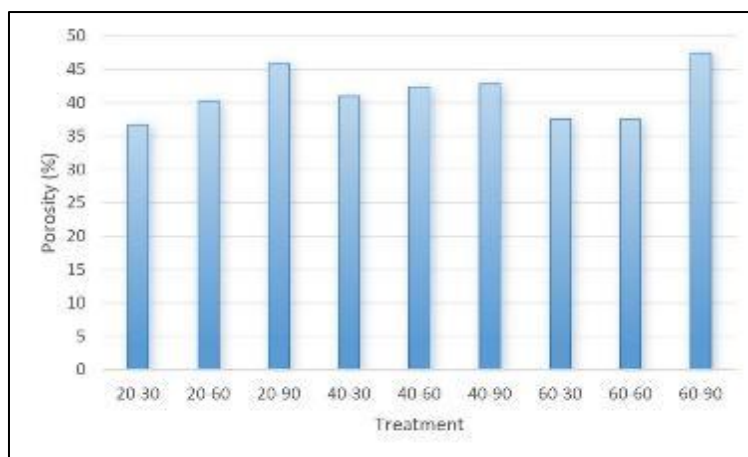


Figure 2 Graph of Coconut Dregs Cell Porosity by Treatment

The FE-SEM images presented in Figure 3 show differences in the surface of coconut dregs cells based on each treatment, with open and irregular pores. The formation of these pores indicates that the PEF treatment successfully opened the lignocellulosic matrix structure, thereby accelerating the degradation carried out by *Agrobacterium* sp. This is characterized by the presence of gaps or holes, small fragments, and cracks. The electroporation effect caused by PEF can break down the cell wall structure, thereby increasing the porosity of coconut dregs cells (17). (18) revealed that electroporation can increase the cell surface area, enhance cell permeability, enlarge pore size, and improve enzyme accessibility—in this case, those produced by *Agrobacterium* sp. The formed pores also indicate that lignin, a component providing rigidity to the cell wall, has undergone partial delignification, making the structure more fragile and open. Enzyme access to hemicellulose and cellulose will be improved in subsequent stages, such as hydrolysis. Biomass with higher porosity typically has lower density due to degradation (16). The delignification process, as shown in the FE-SEM images, is also indicated by a smoother cell surface, suggesting that some lignin has been removed.

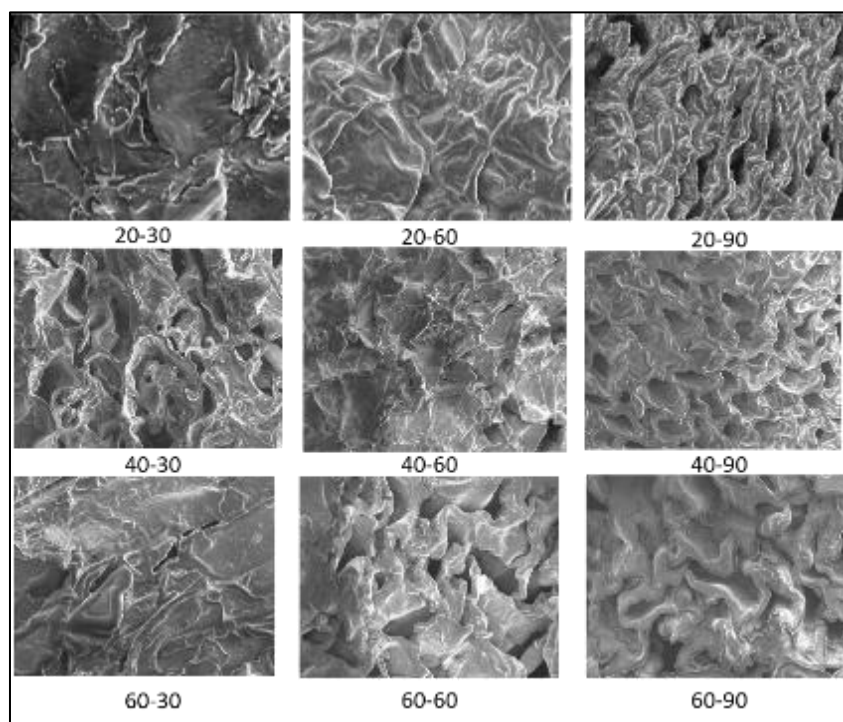


Figure 3 SEM Image of Coconut Dregs Cells after PEF and SSF with *Agrobacterium* sp.

Based on morphological analysis of the coconut dregs cell surface following initial PEF treatment conducted using FE-SEM, and further analysis using Origin Lab software. The observation results are presented in the form of a colored topographic map (Figure 4), where the X and Y axes respectively indicate the spatial position of the coconut dregs cell

surface in micrometers (μm), while the color scale on the right represents surface height (Z) or the intensity of the secondary electron signal, which correlates with the level of roughness and surface topography. Cracks, open pores, and fragmented areas observed in the FE-SEM images appear in the Origin Lab. Software analysis as areas dominated by blue-green hues, representing depressions and new porosity resulting from the opening of the cell wall matrix due to exposure to a high-frequency pulsed electric field (7900 Hz). Generally, blue to green colors indicate lower or concave areas, while yellow to red colors indicate protruding or higher surface regions. These color variations signify morphological heterogeneity resulting from PEF treatment of the biomass lignocellulose structure. The energy induced by the electric field causes cell membrane permeability (electroporation) and disruption of the cell wall microstructure, particularly in areas rich in hemicellulose and lignin (19).

The non-homogeneous structure shown in Figure 4 indicates that the electric field intensity produces an uneven effect, whereby certain parts of the coconut dregs cell surface undergo greater structural damage than other areas. The red-yellow areas represent remaining fragments of lignin or cellulose fibers that are still intact and protruding, while the blue-green areas indicate parts of the cell wall that have undergone degradation or are open, allowing hydrolytic enzymes such as xylanase to interact more easily in the subsequent hydrolysis stage. These results align with previous studies reporting that PEF treatment can increase the porosity and surface roughness of biomass, which in turn enhances the availability of hemicellulose for conversion into furfural (15,20). Thus, the open and irregular morphology observed in these FE-SEM images serves as an indicator of the success of the PEF pretreatment process in optimizing the physical structure of lignocellulosic biomass for subsequent bioconversion processes.

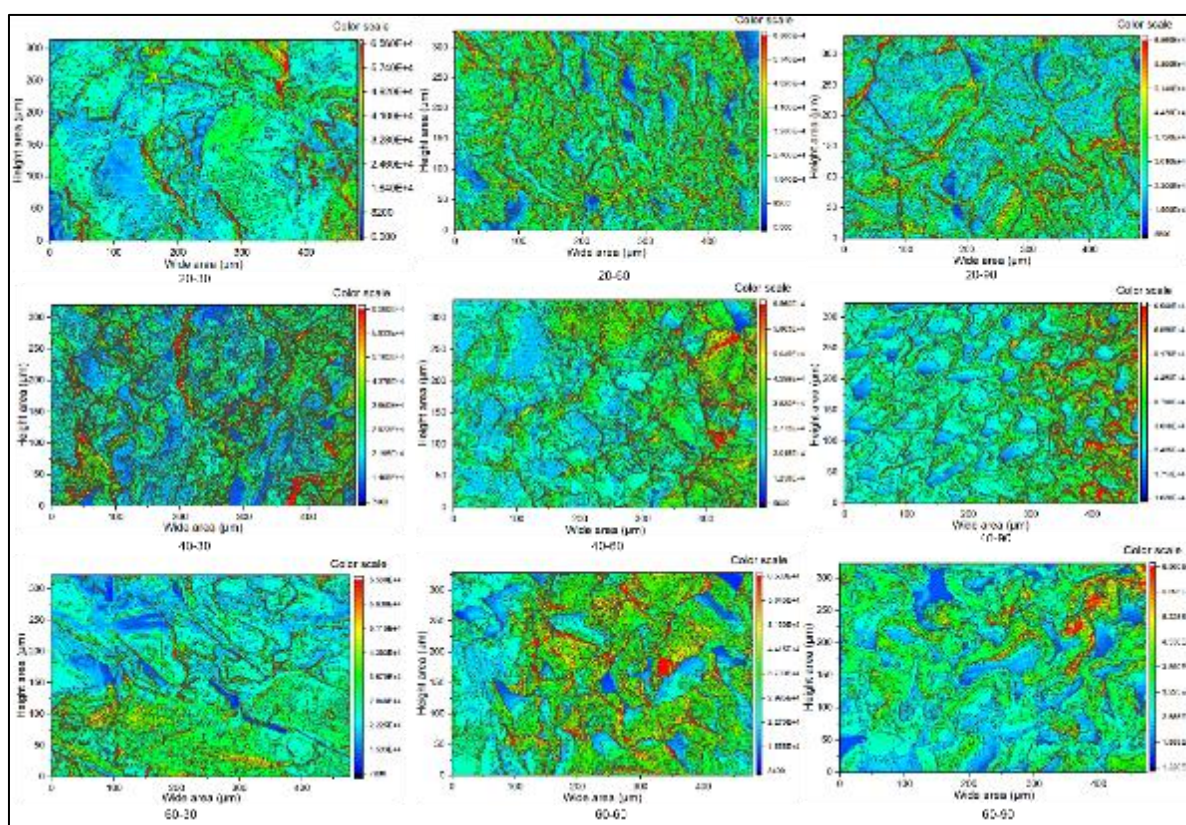


Figure 4 Analysis of FE-SEM Images using Origin Lab. Software

The results of the FE-SEM image analysis using Origin Lab. Software are presented in the form of a color map. The color scale on the right (with different numbers based on treatment) represents the mapping of specific parameters derived from the FE-SEM image. The smooth color gradient from blue to green to red represents the topographic map (height map) of the processed FE-SEM image. The color scale from blue to green, green to red, and red to white represents the surface height (Z) or secondary signal intensity. Where blue indicates areas with low height or intensity (deeper/concave), green indicates flat or intermediate areas, red indicates high areas (protrusions, prominent fibers, or deposited areas), and white indicates the highest areas (topographic maximum). This study aligns with a report by (6,21), which states that PEF treatment followed by biological processes can reduce the compactness of lignocellulosic tissue and increase the specific surface area exposed to hydrolytic enzymes.

4. Conclusion

These findings support the hypothesis that a combination of physical (PEF) and biological (microbial delignification) treatments can produce more effective surface modifications of biomass compared to single treatments. The formation of a more open and porous structure increases the availability of hemicellulose and cellulose, thereby improving the efficiency of enzymatic hydrolysis in the subsequent stage. Although *Agrobacterium* sp. is not as effective as white rot fungi in producing enzymes, PEF is highly effective as an initial treatment for the resulting compost.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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