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Optimization of nanocellulose extraction from *Cocos nucifera* leaves

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Abstract

The most common scenario in agriculture and food manufacturing is they utilize the fruit or stem of the plants but discard the leaves by open burning or disposing them into the river which causes water and air pollution. Nanocellulose fabricated from abundant agricultural waste could be one of the alternative solutions to overcome environmental pollution. In this study, nanocellulose was extracted from the dried leaves of *Cocos nucifera* (Coconut). The process was carried out using formic acid, peroxy formic acid and sodium hypochlorite bleaching. The interaction of three independent variables was studied during the formic acid pulping stage. The optimum condition for the formic acid pulping was set by 85% concentration of formic acid and incubation in the water bath at 70°C for 1 hr. This study showed a simple and inexpensive method to extract nanocellulose. The products provided insight into the kinetic model of nanocellulose and the potential usages of nanocellulose as substitutes for renewable sources of energy and applications in various fields.

1. Introduction

Nanocellulose is referred to as cellulose in its nanostructured nature. Nanocellulose is of expanding enthusiasm for a scope of uses that are significant to the fields of material science and biomedical designing because of its renewable nature, anisotropic shape, tailorable surface science, fabulous mechanical properties, great biocompatibility, and fascinating optical properties (Abitbol, 2016; Evyan *et al*., 2017). Nanocellulose is extended from cellulose chains that are bundled together in a highly ordered region which can be later isolated as nano-particles with high specific surface area (Foster *et al*., 2018).

Highly evolved plants have a cell wall that is characterised by lignocellulose whose physical structure, chemical composition and biological function change according to the species, age as well as type of tissues (Liu *et al*., 2018). Thus, many plants and greens can be extracted to form cellulose and fabricate nanocellulose after treatment. There are lignocellulosic biomasses still in abundance, especially by-products from agriculture and waste from food industries. By utilising or recycling these biomasses, wastage can be minimized and useful products can be produced. In the past, most of the byproducts or waste would be disposed of in the rivers or cleared by open burning. This resulted in serious environmental pollution such as water pollution and air pollution. Nevertheless, this may increase the cost of production indirectly. In previous studies, researchers have fabricated nanocellulose from different parts of plants such as fruit peels from oranges and bananas (Irshad *et al*., 2011), stalks from banana and sugarcane (Pereira and Arantes, 2018), leaves of lemon grass and pineapple and corn cobs (Barhoum *et al*., 2018). In addition, another biomass that is undoubtedly abundant in nature is rice straws which has been widely studied. However, there is still a lack of studies on the extraction of cellulose from coconut leaves compared to leaves from other plants or coconut husks. It is beneficial to extract cellulose from leaves compared to other parts of the plants as it has been proven that the structure of leaves contains the highest number of lignocellulose that are assumed readily available and that can be used as lignocellulosic biomass for the production of nanocellulose.

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According to statistical analysis from the Department of Agriculture (2015), the total area of coconut palm trees in Malaysia was 82,000.5 hectares and in Negeri Sembilan, the total area was 1,576.0 hectares. Coconut palm trees are known to be one of the agricultural biomasses with a high number of lignocellulose content. With the accessibility of biomass, it is entrusted that this innovation is fit for turning the negative cost of biomass into positive-procuring materials. The coconut palm is one of many lignocellulosic biomasses that are readily available and that can be used as lignocellulosic biomass for the production of nanocellulose. Most parts of the plant can be used for different purposes and various products can be obtained from coconut palm trees including tender coconut water, copra, coconut oil and raw kernel.

Nanocellulose is divided into three different types of materials: Cellulose nanocrystals (CNC), cellulose nanofibrils (CNF) and bacterial cellulose (BC). According to Samir *et al*. (2005), cellulose nanocrystals are cellulose structures that are grown under a controlled environment that results in the formation of high-purity single crystals. This type of nanocellulose is produced via mechanical treatment that can either involve or does not involve enzymatic or chemical pre-treatment. Cellulose nanofibrils are defined as a natural, incidental or manufactured material containing particles, in an unbound state, aggregate or agglomerate with particles in the number size distribution range 1 - 100 nm (Evyan *et al.*, 2021). These nanofibrils are considered to be strong and elastic material that has an estimated axial Young's modulus that ranges from 13 to 180 GPa. The bacterial cellulose is secreted extracellularly by some specific bacteria, mainly by Gluconacetobacter strains (Siró and Plackett, 2010; Klemm *et al*., 2011).

The nano-biomaterial has the potential to overtake the use of synthetic nanomaterials in the future due to its features (Dubey *et al*., 2016). The features of properties of nanocellulose include having re-enforcing capabilities, low density as well as being biodegradable (Dufresne, 2013). In addition, nanocellulose has impressive mechanical properties such as low gas permeability and stiffness-enhancing capacity. The potential of this nanocellulose is yet fully known but many useful applications have been implemented with the use of them such as in the production of nano-biomaterials.

2. Materials and methods

The leaves of *Cocos nucifera* were collected from Nilai University, areas of Port Dickson and Nilai 2. The coconut leaves were cut into small pieces, ground to powder (Figure 1) and placed in a conical flask. A mixture of formic acid (HmbG® Chemicals - 45% or 65% or 85%) and hydrochloric acid (CLOROX Regular 5.25%) was added into the flask with a biomass to organic acid ratio of 1:7 and was allowed to boil for 1, 4 and 7 hr(s) using water bath (Techne Tempunit TU-16D). Subsequently, the flask was allowed to cool down to room temperature and the mixture was filtered through the Buchner funnel with the aid of a vacuum pump (PVIV-VAC-PUMP-220). Finally, the biomass was washed using distilled water until the pH reached nearly 7.

Figure 1. *Cocos nucifera* leaves are cut into smaller pieces and ground to powder form.

The biomass was placed inside a petri dish and oven-dried overnight at 65°C. As optimization was done in this step, the Box-Behnken experimental design was used. The filtrate was placed in a conical flask and boiled till dry on a hot plate after filtration via the Buchner funnel was completed. The treated pulps were treated using a mixture of peroxyformic acid (PFA) solution in a hot water bath for 2 hrs at 80°C. Afterwards, the delignified samples were filtered and washed with hot water 65°C. The ratio of cellulosic biomass to PFA was 1:7. This step was done to remove most of the lignin from the sample. The treatment for bleaching was done by adding 35 mL of 5.25% (w/v) sodium hypochlorite to the sample at 70°C for 2 hrs. The mixture was washed with distilled water and oven-dried overnight at 65°C. The homogenization (DAIHAN-brand® HG-15D) step was carried out in a beaker. It was done at an input of 300 W with a speed of 1000 rpm from a titanium alloy rod which was immersed 15 mm under the suspension. This step was done to obtain the nanocellulose in a smaller form. The characterization of samples was done using an optical microscope to characterize the dimension and homogeneity of the nanocellulose.

3. Results and discussion

The gradual colour change can be observed during different stages of the formic acid pulping with the final colour being darker brown compared to the light greenish colour of the untreated biomass. The delignification process can be accelerated with the aid of formic acid (FA) because, in acidic conditions, ether bonds between lignin and hemicellulose can be cleaved faster (Nuruddin *et al*., 2011). The further removal of

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lignin was done using peroxyformic acid (PFA) delignification followed by sodium hypochlorite bleaching. The gradual decolourization of those steps is shown in Figure 2. To further remove the lignin PFA treatment was done. The presence of H_2O_2 in the solution enhanced the delignification procedure because of the joined impact of formic acid as a solvent and peroxy acid as an oxidizing agent to break down the lignin in the biomass sample (Li *et al*., 2012). The total biomass recovered after the formic acid pulping step was 71.3%. This is because some of the biomass was lost during the different stages of delignification, especially during the washing step. The most biomass lost was during the filtration via the Buchner funnel. Some biomass was embedded into the filter paper as shown in Figure 3 and cannot be forcefully removed as the filter paper itself is made up of cellulose and can affect the result of the experiment.

Figure 2. Gradual decolourization through the whole process of PFA treatment, bleaching and homogenization.

Figure 3. Filter paper after the filtration via Buchner Funnel.

Response surface methodology (RSM) was utilized to optimize the yield of cellulosic residue as well as the yield of lignin during the step of formic acid pulping. The effects of temperature (X_t), FA concentration (X_c) and reaction time (X_r) on the yield were studied using Box-Behnken Design (BBD). The model that fits the response variable was a linear response surface model as shown in Figure 4 and Figure 5. The models' coefficients were evaluated via regression analysis and were also tested for significance. The R^2 values indicate that the fitted models accounted for about 99% of the total variations in the experimental data, which means a high significance. The R^2 value obtained for cellulosic residue was comparatively higher than the R^2 value of lignin. The higher the R^2 value, the better the fit of the model to the experimental data (Hasan and Srivastava, 2011). The temperature and the FA concentration had a linear relationship in response to both cellulosic residue as well as the lignin and resulted in a plane response on the graph. It can be concluded that both the cellulosic residue yield and FA lignin yield were solely affected by the FA concentration or reaction temperature. In other words, the two variables did not have an interactive effect on the responses.

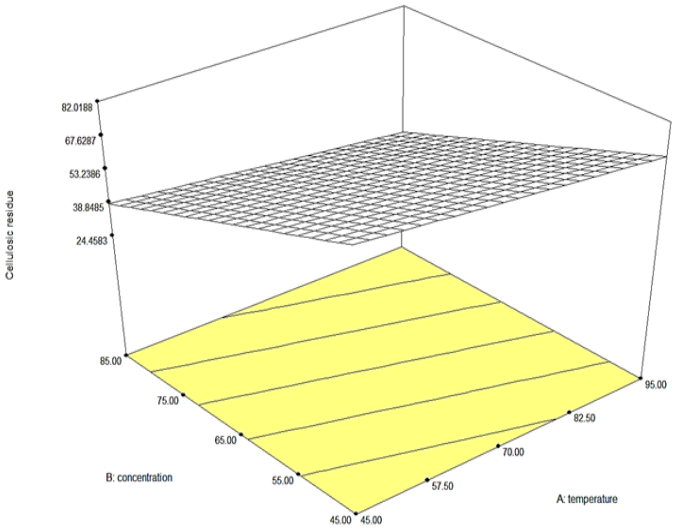

Figure 4. 3-D response surface plot of cellulose residue as a function of FA concentration and temperature with reaction time kept constant at 4 hrs.

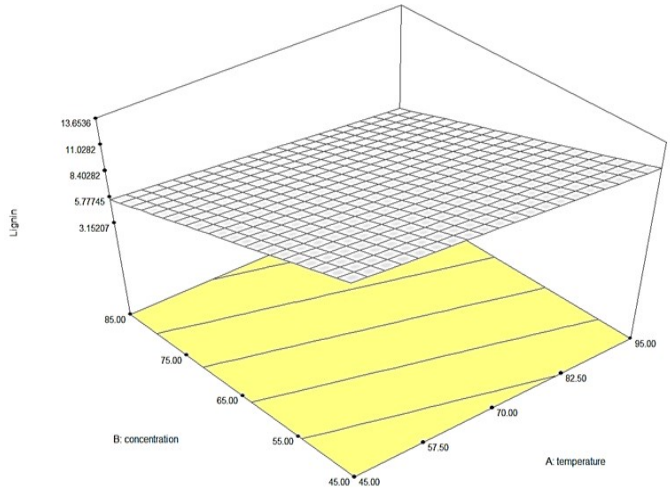

Figure 5. 3-D response surface plot of FA lignin as a function of FA concentration and temperature with reaction time kept constant at 4 hrs.

Optical microscopy was done prior to homogenization as well as after homogenization to observe any differences, if any. Figure 6 shows the optical microscopy of the nanocellulose before homogenization and after it was homogenized.

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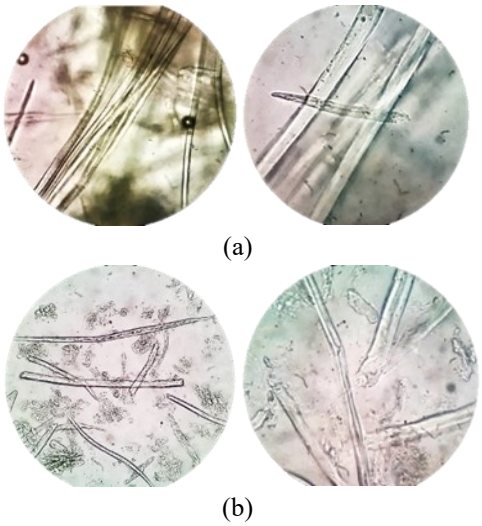
Homogenization is defined as the process of making things smaller, or more uniform (Rohde *et al*., 2015). The cellulosic samples were comparatively longer before homogenization (Figure 6(a)), compared to the samples after homogenization (Figure 6(b)). There was less cell debris in the CNF suspension after homogenization. This is due to the increase in cellulose nanofiber (CNF) disintegration from the cellulose cell walls (Eichhorn *et al*., 2022). Those cells which were subjected to the treatment procedures in this experiment were successfully homogenized.



(a)

(b)

Figure 6. Optical microscopy of the samples: (a) before homogenization and (b) after homogenization at 40× and 100× magnification respectively

4. Conclusion

This study demonstrated a pulping process in which cellulose and lignin can be extracted using formic acid pulping followed by a further delignification and bleaching process. For the formic acid pulping, 61% of the cellulosic residue recovered along with 10.2% of the lignin. The optimum condition for the formic acid pulping was using 85% formic acid concentration and a water bath at 70℃ for 1 hr. The method used in this study is simpler when compared to other methods of extraction of nanocellulose. It can be further studied as the kinetic model for aerogels and hydrogens to be used in major fields such as medicine and renewable sources of energy

Conflict of interest

The authors declare no conflict of interest in this research project.

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