Effect of Pulp Concentration during Cellulase Pretreatment on Microfibrillated Cellulose and Its Film Properties

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Pulp concentration was increased, during preparation of microfibrillated cellulose (MFC), in an effort to improve the efficiency of cellulase pretreatment. It was hypothesized that increased pulp concentration could possibly increase the interactions between cellulase and cellulose, therefore improving the cellulase pretreatment efficiency and benefiting MFC and its film properties. Results showed that higher pulp concentration enhanced the cellulase adsorption ratio from 70% to 90% for pulp concentrations of 2% and 10%, during the pretreatment process. While pulp concentration was changed from 2% to 10% during cellulase pretreatment, the specific area rose from 30.1 m^2/g to 35.5 m^2/g. Compared with the original eucalyptus pulp, the crystallinity degree of different pulp concentration was increased, presumably due to the enzymatic breakdown of amorphous cellulose. In addition, the aspect ratio of MFC rose from 19.1 to 35.5. Concurrently, MFC film properties showed better performance, as the elongation at break increased from 0.75% to 1.95%, tensile strength increased from 15.3 MPa to 33.5 MPa, and oxygen permeability coefficient decreased from 111×10^{-14} $cm^3\cdot cm/cm^2\cdot s\cdot Pa$ to 89.7×10^{-14} $cm^3\cdot cm/cm^2\cdot s\cdot Pa$, reflecting the oxygen barrier properties of MFC film.

Keywords: Cellulase; Pulp concentration; Microfibrillated cellulose (MFC); MFC film

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INTRODUCTION

Microfibrillated cellulose (MFC) is a type of cellulose product that was first developed in the 1980s (Herrick *et al*. 1983; Turbak *et al*. 1983). MFC is attractive as an organic and biodegradable reinforcement in polymer nanocomposites because of its high aspect ratio, good mechanical properties, and ability to form networks (Svagan *et al*. 2007; Bhattacharyya *et al*. 2008; Nakagaito and Yano 2008). In recent years, studies have shown that pure cellulose films have high toughness and strength (Henriksson *et al*. 2008). Because of its favorable properties, such as being a good oxygen and water vapor barrier and its high crystallinity (Aulin *et al*. 2009) and cohesive energy density (Chatterjee and Gupta 2002), it can be used in packaging, paper, medicine, and elsewhere. An improved method for MFC preparation would work with high efficiency, while also being environmentally friendly, controllable, and requiring less energy consumption.

At present, there are two main methods for the preparation of MFC, of which one is mechanical method, such as high-pressure homogenizers (Herrick *et al*. 1983; Turbak *et al*. 1983), micro-fluidizers (Bendahou *et al*. 2010), ultrafine grinders (Jang *et al*. 2013),

and the other involves either chemical or enzymatic pretreatment combined with mechanical treatment (Saito *et al*. 2007).

As a clean and environmentally friendly material, the method of using cellulase pretreatment to produce MFC during the isolation process previously has been investigated with the purpose of using less energy, along with possible reduction in other chemicals (Henriksson *et al*. 2007; Pääkkö *et al*. 2007; Siddiqui *et al*. 2010; Janardhnan and Sain 2011). Improved cellulase pretreatment efficiency is conducive to its commercial use, while cellulase adsorption is a prerequisite step. In recent years, to improve the adsorption efficiency of cellulase on cellulose, researchers have investigated the effect of temperature, pH, substrate concentration, and chemical additives such as ionic solutions, surface active agents, and cationic polymers (Meng and Ragauskas 2014; Wang *et al*. 2015a; Yang and Fang 2015). The addition of Mn^{2+} ions improves the endoglucanase activity and adsorption efficiency (Vasconcellos *et al*. 2016). By adding poly(ethylene glycol) (PEG) 6000 and Tween 80®, the hydrolysis efficiency of cellulase and xylanase can be improved and the amount of cellulase and processing time can be reduced (Li *et al*. 2015). Cationic polyacrylamide (CPAM) can neutralize the charges of cellulose and cellulase, thus eliminating electrostatic repulsion and improving cellulase adsorption (Wang *et al*. 2015b).

Xylanase treatment of brown stock was used to increase the bleaching ability, under low (3% to 4%) pulp concentration by increasing the mixing efficiency between the cellulase and pulp fibers (Sharma *et al*. 2014). Increasing the substrate concentration can enhance cellulase adsorption, improving the hydrolysis efficiency during the cellulase pretreatment process (Várnai *et al*. 2013).

Pulp concentration was increased to improve the cellulase efficiency in the process of dissolving pulp during cellulase pretreatment (Wang *et al*. 2015a). The structure and transport properties of MFC films, including moisture sorption, oxygen permeation, water vapor, and humid oxygen permeation, have been investigated using various methods (Minelli *et al*. 2010). Films produced from quaternized cellulose nanofibers (CNF) exhibit antibacterial properties without any leaching of quaternary ammonium into the environment (Saini *et al*. 2016).

The goal of this work was to prepare MFC using bleached eucalyptus kraft pulp as a raw material for cellulase hydrolysis. Various pulp concentrations were tested during cellulase pretreatment to investigate the influence of concentration on cellulase adsorption, along with the properties of MFC and its films. A scanning electron microscope, a Malvern particle sizer, a Fourier transform infrared spectroscope, and Congo red were used to characterize the MFC properties. The performance of MFC films was further explored through the analysis of elongation at break, tensile strength, air permeability, and light penetration. This paper will offer a theoretical basis for cellulase pretreatment during MFC preparation and examine its effect on MFC and MFC films using various pulp concentrations.

EXPERIMENTAL

Materials

Bleached eucalyptus kraft pulp was supplied by the UPM company, JiangSu, China, and was refined by laboratory-scale Hollander beater (Kumagai Riki Kogyo CO., LTD., Japan) to a beating degree of approximately 30 °SR using a Schopper-Riegler device

(Haage Anagramm Technologien GmbH, Model SR1, Germany) before cellulase pretreatment.

Sukahan cellulase was supplied by Sukahan (Wei Fang) Bio-Technology Co., Ltd. Shandong, China. The cellulase activity was 5898.25 u/g (the cellulase activity per gram), as determined by filter paper activity (FPA), and the cellulase was dissolved in water at 2 g/L prior to use.

Methods

Cellulase adsorption determination

Cellulase adsorption onto cellulose was determined by the Bradford principle, which measures protein concentration (Wang and Xing 2009). Various amounts of pulp (on an oven-dried basis) were suspended in distilled water at a cellulase dosage of 20 u/g (o.d. pulp), placed in 300-mL Erlenmeyer flasks at pH 4.8, and then placed in a water bath at 55 °C for various target times. The samples were then filtered through a funnel, and the free cellulase remaining in solution was determined. The cellulase adsorption ratio was calculated using the following equation reported in the literature (Wang *et al*. 2015b),

$$q = \frac{c_t - c_f}{c_t} \quad (1)$$

where, c_t and c_f are the total and free cellulase concentrations (mg/mL), respectively.

Microfibrillated cellulose preparation

Cellulase pretreatment experiments were carried out in plastic bags, which were placed in a water bath at 55 °C. Various amounts of pulp (on an oven-dried basis) were used in acetic acid buffer solution at pH 4.8 and cellulase (20 u/g o.d. pulp). After 24 h, the pulp was filtered, washed, and collected for PFI mill (Norway pulp and paper institute, Norway) refining. PFI grinding refined the pulp to a beating degree of approximately 85 °SR. Eventually, pulp with a concentration of 1 wt% was diluted by distilled water and then passed 10 times through a homogenization machine (Shanghai Light Industrial Equipment Co., Ltd, Model GJJ, China) (Isogai *et al.* 2011; Liu *et al.* 2014).

Scanning electronic microscopy analysis

The aspect ratio of MFC (the aspect ratio was the length of the fiber to the diameter or equivalent diameter) was determined using a scanning electron microscope (SEM; JEOL IT300LV, Japan) with an accelerating voltage of 10 kV. The beam current (measured between the condenser lens and objective lens) was approximately 1 pA to 1 μA. Prior to the examination, a thin coating (~10 nm) of gold was deposited on the sample using auto fine coater (JEOL JEC-3000FC, Japan), to enhance the conductivity and secondary electron emission characteristics. First, the MFC hydrogel samples were dispersed into water. Second, 1 mL of suspension was diluted with acetone. Thereafter, a drop of 20 μL of *ca*. 0.01 wt% suspension was transferred to a clean glass slide and air-dried overnight at room temperature.

Particle size analysis

Mastersizer 3000 (Malvern institute, Britain) was used to analyze the particle size of a MFC (1 wt%) slurry to establish appropriate test conditions. The samples were

transferred by a dropper to the sample pool until the appropriate shading degree measurements began.

Specific area analysis

The Congo red adsorption method was used to analyze the specific area of the MFC. The method determined the maximal absorbed amount of Congo red by UV measurements (Lavoine *et al*. 2012). Initially, 0.3 g (o.d.) pulp was tested, prepared in a 500-mL beaker, with 100 mL of distilled water. Next, 100 mL of Congo red (0.16 mg/mL) was added and mixed well. After one minute, a 10-mL sample was transferred to a centrifuge tube. After centrifugation, the absorbance on a spectrophotometer was measured. The average value of each sample was measured two times, and the value of deviation was ±0.01m^2/g. The samples were taken to determine Congo red concentration using Langmuir isotherms, according the following equation (2), and the specific area was measured according to the following equation (3) (Spence *et al*. 2010),

$$\frac{[E]}{[A]} = \frac{1}{K_{ad} A_{max}} + \frac{[E]}{A_{max}} \quad (2)$$

where [*E*] is the solution concentration of Congo red at adsorption equilibrium in mg/mL, [*A*] is the absorbed amount of Congo red on the cellulose surface in mg/g (that reached a maximum value equivalent to $A_{max,}$ the maximum adsorbed amount), and K_{ad} is the equilibrium constant,

$$\mathrm{SSA} = \frac{A_{max} \times N \times SA}{MW \times 10^{21}} \quad (3)$$

where A_{max} is the maximum absorbed amount, *N* is Avogadro's constant, *SA* is the surface area of a single dye molecule (1.73 nm^2), and *MW* is the molecular weight of Congo red (696.66 g/mol).

Crystallinity analysis by Fourier transform infrared spectra (FT-IR)

FT-IR was used to study the change in MFC crystallinity. The IR crystallinity index of MFC (Nelson and O'Connor 1964; Kataoka and Kondo 1998) was evaluated as the intensity ratio between IR absorptions at 1427 cm^{-1} and 895 cm^{-1}, which were assigned to the CH_2 bending mode (Liang and Marchessault 1959) and deformation of anomeric CH (Blackwell *et al.* 1970). Dried MFC powder (1 mg) was prepared by mixing with KBr (160 mg) and pressing into pellets. The pelletized sample was then set on the infrared spectrometer in the range from 800 to 4000 cm^{-1}. The average value of each sample was measured three times, and the value of deviation was ±0.1~1.5%.

Properties of MFC film

A set of five different MFC (1wt%) film sample types was prepared by casting, while films were prepared at 45 °C and 50% RH for 48 h (Minelli *et al*. 2010). The films were conditioned at 23 °C and 50% RH one day before measure properties of MFC films. Tensile properties of MFC films were based on GB/T 1040.3 (2006) and tested by a universal testing machine Instron 3369 (Instron, USA) and a gauge with a precision of 0.01 mm was used to test its thickness. Air permeability of MFC films was based on GB/T 1038

(2000) and tested with an air permeation tester GDP-C (Brugger, Germany). Five replicates were used for these measurements.

RESULTS AND DISCUSSION

Effect of Pulp Concentration on Adsorption Performance

Figure 1 shows that cellulase adsorption efficiency increased with increasing pulp concentration, with a cellulase adsorption rate increase from 70% to 90% when pulp concentration was increased from 2% to 10%. This suggests that higher pulp concentrations improve the interaction of cellulase and cellulose. Similarly, Wang *et al*. (2015a) using prehydrolysis kraft-based dissolving pulp observed that increasing pulp concentration could improve cellulase adsorption ratio from 59% to 74% when pulp concentration was increased from 3% to 10%, the purpose of which was to improve dissolving pulp properties.

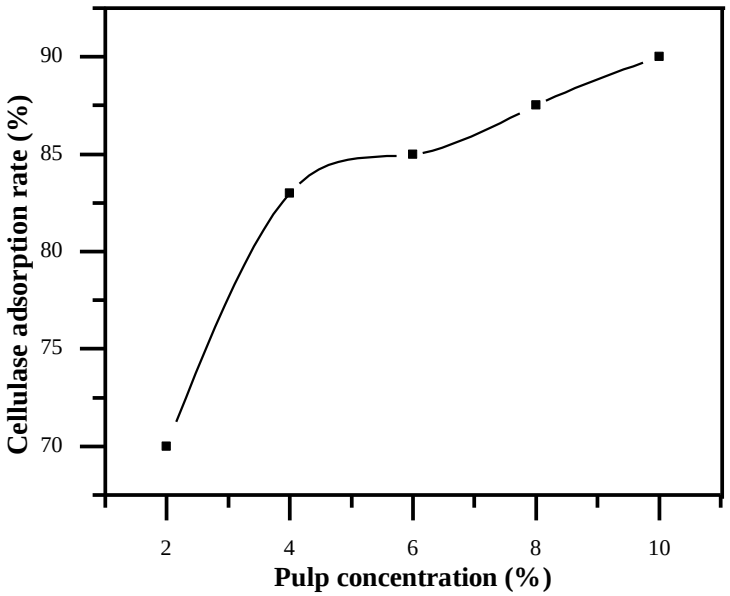

Fig. 1. Effect of different pulp concentrations on the adsorption of cellulase on cellulose

Effect of Pulp Concentration on MFC Performance

MFC with a solid content of 1% was prepared using various pulp concentrations during pretreatment. Figure 2 shows its properties in detail. The average particle size was reduced from 52.4 to 40.8 μm with increased pulp concentration from 2% to 10%. This indicates that the pulp concentration had a considerable impact on the MFC particle size, primarily as a result of the cellulase pretreatment, where long-chain cellulose molecules are truncated, resulting in large quantities of fine fibers. Figure 2 also shows that the specific area of MFC increased with an increase in pulp concentration, which is due to the decrease in particle size. Table 1 shows its crystallinity degree. In Table 1, the values increase compared with the original eucalyptus pulp, which had a crystallinity degree of 73.4%. This was attributed to the ability of cellulase treatment of amorphous cellulose to decrease the amount of cellulose microfibrils. As can be seen in Table 2, with the pulp

concentration increased from 2% to 8%, the crystallinity degree decreased. This is due to the fact that a high shearing action during homogenization process may lead to a possible peeling-off effect of the cellulose chains on the surface of the crystallites. But when the pulp concentration was 10%, the crystallinity degree was raised; this is presumably because cellulase treatment of amorphous cellulose was greater than the homogenization peel-off cellulose chains process. The crystallinity degree of the cellulose was increased, compared with the original eucalyptus pulp, so the bonding strength between the celluloses and the stiffness of the prepared MFC was enhanced.

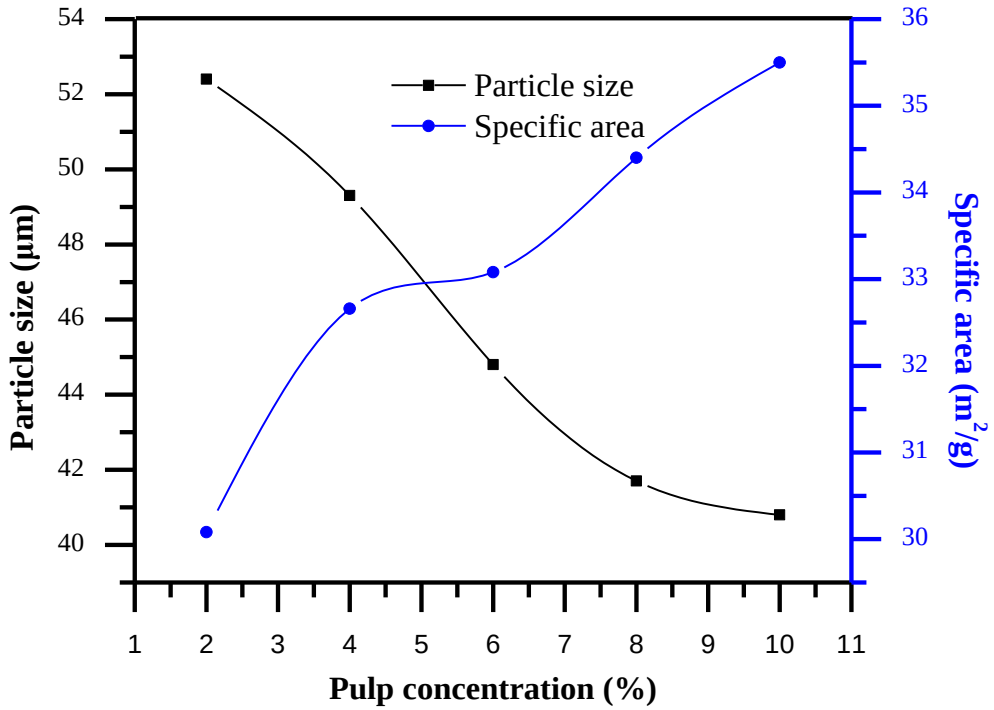

Fig. 2. Properties of MFC from various pulp concentrations

Table 1. Crystallinity Changes with Pulp Concentration

Pulp concentration for 1 wt% MFC (%)	2	4	6	8	10
Crystallinity degree of MFC (%)	86	85.6	85.4	81.1	90.7

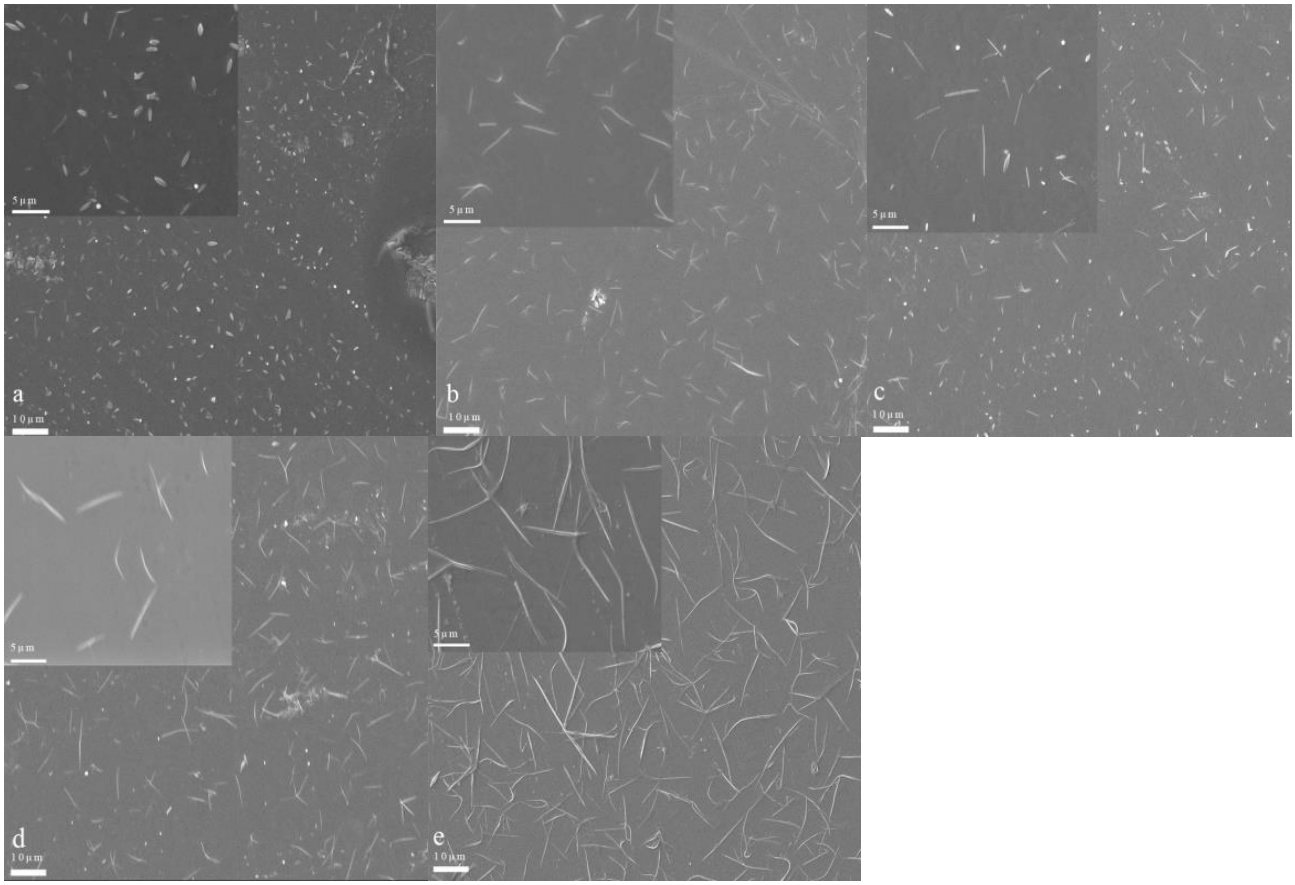

Fig. 3. SEM micrographs of MFC prepared from various pulp concentration (a. 2%, b. 4%, c. 6%, d. 8%, e. 10%)

Figure 3 shows images of MFC under magnifications of 1000× and 4000×. It can be seen that the fiber fragments decreased with increasing pulp concentration. This can be explained by the fact that the increased concentration of pulp improves the peel-off effect of the cellulose cell wall during the cellulase pretreatment process and decreases the fiber fragment content during the isolation process. The aspect ratio of MFC increased with increasing pulp concentration, as shown in Table 2, which value was measured three times. When the pulp concentration was increased from 2% to 10%, the aspect ratio of MFC rose from 19.1 to 35.54. This is accordance with what was found in enzyme-assisted isolation of MFC from date palm fruit stalks (Mohammad *et al*. 2014), where it was found that fiber length was 610 to 810 μm and width was 23.4 μm (aspect ratio 26.1 to 34.62). Accordingly, a pulp concentration of 10% can more easily form a stable network structure of MFC during the preparation process.

Table 2. Effect of Pulp Concentration on the L/d of MFC

Pulp concentration (%)	2	4	6	8	10
Aspect ratio	19.1	20.54	19.59	23.35	35.54

Effect of Pulp Concentration on the Properties of MFC Films

Figure 4 and Table 3 show the properties of MFC films (film thickness was approximately 50 μm) prepared using the various concentrations of pulp in the pretreatment process.

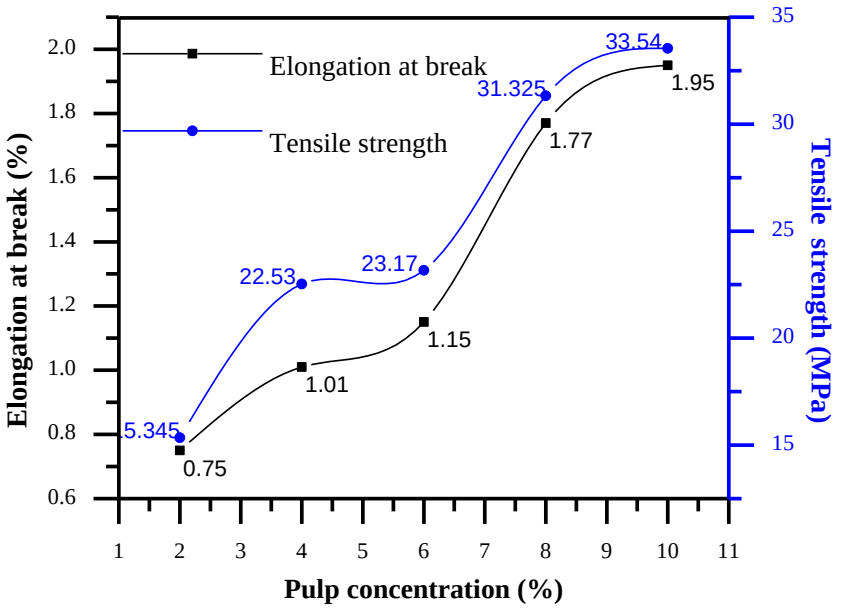

Fig. 4. Properties of MFC films prepared by pretreatment with various pulp concentrations

Table 3. Permeability Coefficient of MFC Films

Pulp concentration (%)	2	4	6	8	10
Oxygen permeability coefficient×10^{-14} (cm^3·cm/cm^2·s·Pa)	111	106	100	94.5	89.7

The results of elongation at break and tensile strength as a function of the changing pulp concentration are summarized in Fig. 4. The elongation at break and tensile strength improved with increasing pulp concentration. Elongation at break and tensile strength achieved the maximum value at a pulp concentration of 10%. This observation can be explained by the larger specific area of the cellulose in the MFC films. Therefore, the increase in the pulp concentration was beneficial for producing better MFC films with better performance. The results are closely related to the specific area and the aspect ratio of MFC, as the larger specific area and the aspect ratio of MFC are favorable to forming a stable network MFC films.

According to the results in Table 3, it was concluded that the lowest permeability coefficient occurred at a pulp concentration of 10%. Notably, the oxygen permeability coefficient and oxygen barrier properties are inversely proportional. Consequently, the values in Table 4 indicate excellent oxygen barrier properties of MFC at higher pulp concentration. This observation is in line with the specific area and aspect ratio data, due to the increased specific area and aspect ratio making it easier to form a dense network structure. Thus, with the transformation of cellulose into MFC film, the material still remains its excellent properties, the network of which presents a particularly compact and stiff structure.

CONCLUSIONS

1. Cellulase adsorption was enhanced noticeably with increasing pulp concentration.
2. When the pulp concentration was 10%, the aspect ratio and specific area of MFC were larger than those at 2% pulp concentration.
3. The FT-IR spectrometry analysis revealed that cellulase pretreatment did not affect the crystallinity of the MFC, significantly, when the pulp concentration from 2% to 8%, the high shearing action homogenization process played dominant role, but when the pulp concentration of 10%, the cellulase hydrolyzes amorphous surface chains played a dominant role.
4. At the pulp concentration of 10%, the elongation at break and tensile strength of MFC film increased from 0.75% and 15 MPa to 1.95% and 33.54 MPa, respectively, compared with those at the pulp concentration of 2%. Under the same conditions, the oxygen permeability coefficient decreased from 111×10^{-14} to 89.7×10^{-14} $cm^3\cdot cm/cm^2\cdot s\cdot Pa$.

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