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MATERIALS BALANCE IN EXTRACTION OF NANOCELLULOSE FROM FOREST PIONEER SPECIES

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INTRODUCTION

Forest pioneer species are species that first appear, easily establish, and grow in open, highly degraded sites (Mudappa D & Raman TRS 2010). The sites include forest areas, and areas after land disturbances such as logging, fire or abandoned agricultural fields. Pioneer species can also germinate when a gap is created in a dense canopy due to large forest trees falling down and bringing together nearby vegetation. Canopy disturbances such as tree falls, lightning strikes, landslides, pathogens can also contribute to pioneer species germination (Goodale et al. 2012). Some tropical forest pioneer species are *Macaranga, Alstonia*, *Dillenia*, *Leucaena* and *Acacia*. Pioneer species are normally fast-growing and have short life span. The properties of some of these species have been studied, but due to wide spread and non-localised growth of these pioneer species, harvesting and utilizing them for commercial purpose are difficult. An effort is taken by Forest Research Institution Malaysia (FRIM) to establish plantation plots of tropical forest pioneer species such as *Leucaena* as a means to commercialize them.

Cellulose is the basic building block in plants. It has been used in various forms for thousands of years for various applications due to its strength and availability. With an estimated in excess of 75 billion tonnes production globally (Habibi et al. 2010), cellulose continues to attract more research interests. Nanocellulose, a new form of processed cellulose (one thousandth meter smaller) has attracted research attention in the past decade. Besides the availability of cellulose worldwide, a strong interest in nanocellulose is also because of increased demand for biodegradable and sustainable products and also due to its exceptional characteristics that offer vast potential across many application fields (Latifah & Sharmiza 2017). Nanocellulose is the material extracted from native celluloses which can be obtained from plants (wood and non-woods), marine animals (tunicate), algae and bacteria. Some nanocelluloses have been extracted from various resources which include bleached softwood pulp (Araki et al. 1999; Beck-Candanedo et al. 2005), eucalyptus (De Mesquita et al. 2010), sisal (Siqueira et al. 2009) and tunicin (Favier et al. 1995). The potential of nanocellulose extraction from *Acacia mangium*, a tropical pioneer species has also been studied (Latifah & Sharmiza 2017).

Material or mass balance is an application of the law of conservation of mass, which states that mass can neither be created nor destroyed. One kg of total material input will only give one kg of total output, i.e. total mass of input = total mass of output. A material balance is an accounting for materials. Thus, material balances are often compared to the balancing of current accounts. They are used in the industry to calculate mass flow rates of different streams entering or leaving chemical or physical processes. In simple language, a mass that enters a system must, by conservation of mass, either leave the system or accumulate within the system. Figure 1 illustrates the simplified material balance of a process based on two (2) input materials M_1 and M_2 and two (2) output materials O_1 and O_2. The process can either be reactive or non-reactive. In a reactive process, reactants M_1 and M_2 may react and form products O_1 and O_2. Unreacted M_1 or M_2 can also leave the process. In carrying out a material balance, all participating elements and non-participating elements (such as inert gas or water) are usually taken into consideration.

Commercial computer simulation or modeling software such as Aspen Plus or HYSYS can be used to carry out material balance. This works highlights a material balance carried out in the nanocellulose extraction process based on two different routes: acid hydrolysis and enzymatic hydrolysis. The process boundary covers pulping, bleaching, hydrolysis and mechanical treatment.

Figure 1 Simplified material balance

MATERIALS AND METHODS

Determination of chemical compositions of wood

Major wood components such as celluloses (hemi and alpha) and lignin were determined on air-dry wood meal which passed through a BS 60 mesh sieve (250 microns) according to methods listed in Table 1. The alpha-cellulose content determined will serve as a guide on the expected pulping recovery. The sieved wood meal was extracted using the Soxhlet extraction apparatus with 190 ml ethanol-toluene extraction for 6–8 hours as a pre-requisite step for cellulose and lignin contents determination.

Table 1 Methods for chemical analysis

Properties	Method
Preparation	TAPPI T257 cm-02 Sampling and Preparing Wood for Analysis
Ethanol-toluene solubility	TAPPI T204 cm-97. Alcohol-benzene Solubility of Wood
Acid insoluble lignin	TAPPI T222 om-02. Acid-insoluble lignin in Wood and Pulp
Holocellulose	Wise, L.E., Murphy, M. & D'Addieco, A.A. 1946. Chlorite holocellulose: Its fractionation and bearing on summative wood analysis and on studies on the hemicelluloses. *Paper Trade Journal* 122(2): 35-43
Alpha-cellulose	TAPPI T203 om-93. Alpha-, Beta- and Gamma-cellulose in Pulp

Preparation of pure cellulose at laboratory scale

1. Preparation of chemical pulp

   Logs from forest pioneer species were processed into sawn timbers at FRIM sawmill before being chipped and screened into various sizes. The wood chip portion between 20 and 25 mm width and 3 to 8 mm thick was taken for chemical pulping. The moisture content of the wood chip was determined by drying the chips in an oven at 105±2 °C overnight. Two types of chemical pulping processes were considered, depending on the subsequent nanocellulose extractions to be carried out. For acid-based hydrolysis, kraft or sulphate pulping process is employed whereas for enzymatic hydrolysis soda pulping is preferred. A 1000g oven-dry equivalent of wood chip was pulped in a 16L rotary digester using a wood to liquor ratio of 1 to between 6 to 8, depending on the density of wood (Figure 2). For this work, the ratio of 1:6 was used. The amount or concentration of alkali ranged between 16% and 25% which produced pulp with kappa number around 20. The pulping temperature was set at 170 °C with the time taken to reach the temperature of 60 minutes. The pulping temperature was retained for another 120 minutes. The resulting pulp was thoroughly washed and screened using Sommerville fractionator to remove impurities and shives or unliberated fibre bundles. The pulping yield or recovery and Kappa number were determined according to T236 om-99 test method. The residual black liquor contains dissolved cellulose, lignin, extractives, unreacted chemicals and water.

Figure 2 Pulping in 16L rotary digester

2. Pulp bleaching

The pulp was next bleached using a 5-stage elemental chlorine free (ECF) DEDED process with 3% sodium chlorite ($NaClO_2$) for D stages and 2% NaOH for E stages. All bleaching stages were carried out at 70°C with reaction time of 120 minutes for stage 1, 60 minutes for stages 2 and 4 and 90 minutes for stages 3 and 5 (Table 2). A 100g oven-dry equivalent of pulp was bleached in polyethylene (PE) bags for each set based on the steps shown in Figure 3. Acetic acid (CH_3COOH) was also used during the D stage to improve colour removal.

Table 2 Bleaching condition

	D1	E1	D2	E2	D3
Chemical & charge	ClO_2 (3%) CH_3COOH (2%)	NaOH (2%)	ClO_2 (3%) CH_3COOH (2%)	NaOH (2%)	ClO_2 (3%) CH_3COOH (2%)
Pulp consistency (%)	10	10	10	10	10
Time (min)	120	60	90	60	90
Temperature (ºC)	70	70	70	70	70

Figure 3 Bleaching process of cellulose

Extraction of nanocellulose

i. Acid-based hydrolysis

50g of bleached cellulose was mixed with 440 mL 64% sulphuric acid at 45 °C for 40 minutes with constant stirring. Following acid hydrolysis, the suspension was diluted two-fold with cold deionized water (4 °C) followed by centrifugation. Three successive centrifugations were then carried out at 10,000 rpm for 20 min each to remove residual acid. After centrifugation, the suspension was dialysed to neutrality using cellulose membrane tubing (MWCO 12K–14KDa diameter 48 mm) against deionised water. The suspension was then sonicated for 25 minutes at 20% amplitude using Qsonica Sonicator (Q700) to homogenize the suspension and separate aggregates prior to filtration through a fritted-glass filter (Grade 2). The resulting NCC was freeze-dried to produce white NCC powder.

ii. Enzymatic hydrolysis

A 5g bleached cellulose was placed in an Erlenmayer flask. A 100 mL 0.05 M sodium acetate buffer solution was mixed with the enzyme solution and added into the flask. The flask was placed in a shaking waterbath at 150rpm and a pre-determined temperature and left for 72 hours. Upon completion of the hydrolysis time, the mixture was centrifuged at 10,000 rpm for 20 minutes. The

supernatant was refrigerated before further sugar content (using HPLC) and enzyme activity analysis were carried out. Boiling deionised (DI) water was added to the cellulose residue and left for 10–15 minutes to terminate any further hydrolysis. Three rounds of washing were carried out with DI water to remove residual enzyme. The cellulose was dried and weighed at this point to determine the hydrolysis recovery.

iii. Mechanical treatment

To assist cellulose liberation, the cellulose residue was transferred into a homogenizer or PFI mill for shear homogenization. The suspension was then sonicated for 25 min at 20% amplitude using Qsonica Sonicator (Q700) to homogenize the suspension and separate aggregates prior to filtration through a fritted-glass filter (Grade 2) to remove fibre lumps. The suspension was transferred into a Schott bottle before freeze drying. The yield or recovery was calculated based on the starting material which was determined by drying a certain amount of suspension in the oven at 105±2 °C for a minimum of 4 hours.

RESULTS AND PUTTING IT ALL TOGETHER

The chemical compositions of major wood components for some forest pioneer species are shown in Table 3. The percentage contents of holocellulose for these species vary between 68.7 and 78.5%. The alpha-cellulose content ranges between 39.1 and 50.3%, whereas lignin content is between 23.5 and 29.7%. The extractives for these species are between 0.1 and 2.4%. Since the methods used for chemical analysis are gravimetric (weight difference before & after extraction), an error of up to 10% is quite common. For the purpose of this work, a correction factor of 0.936 was applied to account for the gravimetric analysis error. Table 4 shows the pulping results in term of recovery and kappa number for two types of pulping of *Macaranga tanarius*. The recovery of both pulping processes was quite low (below 40%) even though the alpha cellulose content is higher. This could be due to over-delignification for both processes as confirmed by the low kappa numbers calculated (14 and 18 for soda pulping and sulphate pulping processes, respectively). After leaving the digester and removal of black liquour, the pulp contained about 70% moisture. Table 5 shows the material balance on each pulping, bleaching, hydrolysis and mechanical treatment processes. Enzymatic hydrolysis was carried out using 2.5% enzyme dosage. For simplification, this work differentiates the solid and liquid streams as Output 1 and Output 2. Each process may contain more than one process unit or equipment. A material balance for each equipment can also be carried out. The process flow and material balance for enzymatic nanocellulose extraction from *Macaranga tanarius* is shown in Figure 4.

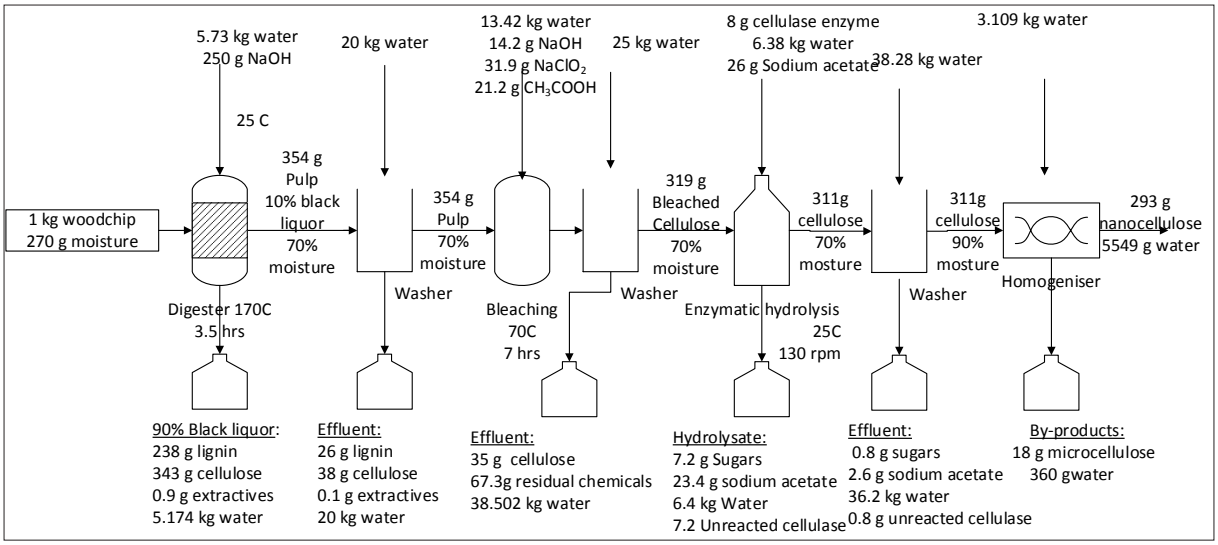

Figure 4 The process for material balance of enzymatic nanocellulose extraction process from *Macaranga tanarius*

Table 3 Chemical compositions of some forest pioneer species

Species	Holocellulose	Alpha-cellulose	Lignin	Extractives	Reference
Macaranga tanarius	78.5	44.0	28.2	0.1	This work
Acacia mangium	77.5	50.3	23.5	2.4	Mohd Nor 1991
Dillenia reticulata	70.9	47.4	24.1	0.8	Peh et al. 1986
Alstonia	65.4	39.1	29.7	1.6	Peh et al. 1986

Table 4 Pulping, bleaching and enzymatic hydrolysis results of *Macaranga tanarius*

	Soda pulping	Sulphate pulping
Active alkali	25 %	18 %
Recovery	35.4%	37.8%
Kappa number	14	18
Bleaching recovery	90%	90%
Hydrolysis recovery	97.5%	95.8%
Mechanical treatment recovery	94.2%	97.5%

Table 5 Summary of materials input and output streams (soda pulping process)

Process	Material	Input (g)	Output 1 (solid) (g)	Output 2 (liquid) (g)	Total output (g)
Pulping (Digester + washer)	Wood	1,000			
	Cellulose	735	354	381	735
	Lignin	264	-	264	264
	Extractives	1	-	1	1
	Water	26,000	826	25,174	26,000
	Chemicals (NaOH)	250	-	250	250
	Total output stream mass (g) =		1180	26,070	27,250
Bleaching (Reactor + washer)	Cellulose	354	319	35	354
	Water	39,246	744	38,502	39,246
	Chemicals (NaOH, CH_3COOH, $NaClO_2$)	67.3	-	67.3	67.3
	Total output stream mass (g) =		1063	38,784.3	39,847.3
Hydrolysis (reactor + washer)	Cellulose	319	311	-	311
	Water	45,404	2800	42,604	45,404
	Cellulase enzyme	8	-	8	8
	Sodium acetate	26	-	26	26
	Sugar	-		8	8
	Total output stream mass (g) =		3111	42,646	45,446
Mechanical treatment	Cellulose	311	293	18	311
	Water	5,909	5,567	342	5,909
	Total water input = 116,559				

CONCLUSION

A simple or meticulous material balance can be carried out for wood-based product processing. In this work, a material balance was shown for nanocrystalline cellulose extraction process from *Macaranga tanarius*. The process include pulping, bleaching, hydrolysis and mechanical treatment. Based on the information collected in this work, the amount of wood, chemicals and water can be determined. Close to 117 litres of water was needed to process 1 kg of *Macaranga tanarius* wood into nanocellulose. Thus, the materials costs for upscaling can be estimated. These steps can be applied to other wood or non-wood species, provided the information required are available. The information can be obtained experimentally or from the literature.

Of course, the next step will be calculating the energy balance which requires the process parameters' information such as pulping, bleaching and hydrolysis temperature, pulping, bleaching and hydrolysis time as well as the power rating of all equipment used. By establishing the energy balance to complement material balance, the operating cost of the process can be determined.

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Forest pioneer species such as *Macaranga*, *Leucaena* and *Acacia* have been studied by FRIM as new potential sources for the wood industry. These species are normally fast growing and have short life span. Products such as furniture, wood composites, pulp and paper have been developed using these species. Converting these species into new emerging products such as nanocellulose were also conducted. A materials balance demonstrates the law of mass conservation and can be used to estimate the amounts of raw materials used and the quantities of products and by-products in a process. In this work, a materials balance was conducted on an enzymatic nanocellulose extraction process inclusive of pulping, bleaching and hydrolysis steps. Based on 1 kg of *Macaranga tanarius* wood chips, 293 g nanocellulose was produced using 250 g sodium hydroxide for pulping, 67.3 g bleaching chemicals, 8 g cellulase enzyme and 26 g sodium acetate as hydrolysis buffer. The materials balance conducted shows that the whole process requires 117 litres of water.

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