Preliminary Study on Nanocellulose Production in Local Seaweeds

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Abstract
Seaweeds are sessile or immobile organisms that are equipped with remarkable cell wall strength that helps them to withstand the strong and uncontrollable sea waves and unfavourable condition in their habitat. With application of mechanical and chemical procedure, cellulosic materials from seaweed can be transformed into nanocellulose which is highly valuable in industry. This research aims to investigate the potential of local seaweeds collected in Port Dickson, Teluk Bidara Trengganu and Langkawi for nanocellulose production. The collected seaweeds were first morphologically identified, cellulose particles were prepared from the seaweeds and the obtained particles were characterised using particle size analyser (PSA). Six species of seaweeds identified were *Caulerpa corynophera*, *Caulerpa microphysa*, *Dictyota dichotoma*, *Laurencia intricata*, *Sargassum siliquosum* and *Padina minor*. For the preparation of cellulose particles, the smallest particles obtained was 1.259 µm from *C. microphysa* while the largest was 275.423 µm from *D. dichotoma.* Generally, the cellulose particles obtained were not in nano size. With improvement of nanocellulose preparation procedure, this preliminary study marks the beginning of bigger research on local seaweeds that have high potential for production of nanocellulose.

Keywords: Industry, Local, Morphologically, Nanocellulose, Seaweed

Introduction
Nanocellulose is cellulosic material that transforms into cellulose nanofibers (CNFs) and cellulose nanocrystals (CNCs) after mechanical and chemical procedures being applied to it (Mishra *et al.,* 2018). Since the principal constituents of fibers are cellulose, plants part such as wood pulp, cotton fibers, bagasse, straw, ramie, sisal, flax and hemp become the primary source of nanocellulose (Mishra *et al.,* 2018). Due to their abundance in nature, availability of the industrial facilities as well as low-cost, plants stand as important source for cellulose production (Das *et al*., 2010). Besides plants, another three most well-known sources of cellulose are algae, bacteria and tunicates (George & Sabapathi, 2015).

Seaweeds, the common name for marine macroalgae are primary producers, shelter, nursery grounds and sources of food for marine life which contributes significantly to economy. They can be found living in all coastal regions and all climate zones ranging from icy polar area to warm tropics (Kumaresan *et al.,* 2015). Seaweeds are said to be the most suitable and practical source for cellulose extraction because their cell wall are mainly made up of cellulose fiber (Mishra *et al.,* 2018). Meaning to say, extraction of cellulose from seaweeds are highly promising and the possibility to extract desires amount of cellulose from them is bigger. According to Baghel *et al*. (2021) the cellulose content of different green, red and brown seaweeds was found to be in the range of 1.5 to 34% of their dry weight.

Furthermore nanocelluloses prepared from various seaweeds are proven to have high degree of crystallinity which is approximately 58 - 99% with diameter size in the range of 2.3 – 40 nm (Baghel *et al*., 2021). Presence of thick microfibrils is believed to be responsible for this good characteristic (Mishra *et al.*, 2018). In terms of end product, the characteristics of nanocellulose depends on the biosynthesis process occurring in different species of algae. The protocols used in producing the nanocellulose will greatly influence the end product in terms of composition thus affecting their strength, characteristics and further application.

Nanocellulose is a highly valuable material that can be used in many fields such as construction, pharmaceutical, biomedical and food industry (Klemm *et al.,* 2011). In biomedical sector, ongoing research is carried out to find out the application of nanocellulose at molecular and macroscopic level. The former focuses on tissue bio scaffolds for cellular culture, drug excipient and drug delivery, and immobilization and recognition of enzyme and protein while the latter focusses on blood vessel and soft tissue substitutes, skin and bone tissue repair materials, and antimicrobial materials (Lin & Dufresne, 2014). In the construction field, nanocellulose can be used as filler materials for reinforcement purpose substituting the artificial filler such as glass, carbon and aramid (Trache *et al.*, 2017). Due to the usages of nanocellulose in various industries, the demand for this material is steadily increasing (Zhang *et al*., 2021). Hence this study aims to investigate the potential of local seaweeds for nanocellulose production.

Methodology

Collection and preparation of seaweed samples

Seaweeds were collected from beaches in Port Dickson, Negeri Sembilan, Teluk Bidara Beach, Dungun and Fisheries Research Institute (FRI) Langkawi. The seaweeds collected were washed repeatedly with distilled water until contaminants and dirts were completely removed from the surface of fibers. Then, around 50 g of the samples were dried in an oven at 55^{o}C for 2 days and ground to fine powder with an electrical blender.

Morphological identification of seaweeds

Seaweed samples were identified into their respective species and genus by means of morphological characteristics.

Preparation of cellulose particles

Cellulose particles were prepared following the steps suggested by Singh *et al*. (2017) with minor modifications. First, the seaweed powder samples were subjected to pre-treatment with 2.5M NaOH under microwave irradiation for 15 minutes at 100˚C. Dewaxing of biomass was induced through simultaneous microwave heating. Slurry obtained was then allowed to be cooled at room temperature and filtered through the vacuum filtration method using Whatman filter paper. The slurry rendered as filter cake was washed with hot distilled water few times until neutral pH was achieved. Distilled water was used through out all experimental process. The samples were then dried in oven at 55˚C. Next, the alkali pre-treated samples were bleached using 35% hydrogen peroxide for 4 hours at 55˚C for complete delignification. The obtained samples were washed repeatedly with hot distilled water, oven-dried at 55˚C, and stored in an airtight container for further characterization. The bleached samples were further subjected to hydrolysis using 1M sulphuric acid and 1M 1-ethyl 3-methylimiddazolium chloride using ultra sonication (450 W) for 45 minutes at 80˚C. The resulting samples were freeze dried for characterization.

Characterization of cellulose particle using particle size analyzer

Characterization of cellulose particle was done using Hydro 2000 MU Malvern Mastersizer (United Kingdom) following manufacturer's manual. The method to handle the machine was divided into three parts which were preparation, technical setting and sample detection. For preparation part, the Mastersizer program in the desktop was first opened. Preparation step is important to clean the system. It started by switching on both laser diffraction and water pump unit. Distilled water in the beaker located at the water pump section was discharged. Then, the beaker was filled with distilled water up to 600-700 mL and placed at the pump section with turbine dunked inside. The pump was set at 3000rpm and the green button labelled "PRESS TO DRAIN, RELEASE TO FILL" was pressed.

The machine was let run for about 1 to 2 minutes to make sure all the particles that went or passed through the lens will come out and cleaned by the water. Next, the green button was pressed once again. The preparation step was repeated twice to make sure the system was really clean before detecting the samples. After that, the distilled water was changed again and the pump speed was set to 2000 rpm, required speed for sample detection before adjusting the technical setting on PC. Technical setting on the PC and PSA machine as well as sample detection steps was done strictly following the Mastersizer User Manual. Normal distribution graph obtained at the end of the procedure was analysed.

Results and discussion

Identification and characterization of seaweeds

Identification of seaweeds was done through observation of their morphological characteristics such as the colour, holdfast, stipe and blade. According to John *et al.* (2002) and Chen *et al.* (2016), seaweeds can be divided into three main groups based on their pigmentation which are green algae (Chlorophyta), red algae (Rhodophyta) and brown algae (Phaeophyta). The taxonomical classification of seaweeds can be done by identifying their morphological features, locations in which they are living, water quality parameters as well as the aquatic organism associated with their habitat (Othman *et al.*, 2018). In terms of structure, general macroscopic algae are expected

to have a holdfast, a stipe and a blade or frond. However, identification of seaweeds is difficult due to simple morphologies, phenotypic plasticity and convergent evolution (Giuseppe, 2012).

Six species of seaweeds were identified by observing their mophological characteristic (Figure 1). Two species of *Caulerpa* namely *Caulerpa corynephora* and *Caulerpa microphysa* and one species of *Sargassum* ie. *Sargassum siliquosum* were identified. *Caulerpa* is green seaweed under the member of Caulerpaceae meanwhile *Sargassum* species is brown algae categorised under family of Sargassaceae (Asmida *et al.*, 2017). The rest of the seaweeds collected in this study were identified as *Dictyota dichotoma*, *Laurencia intricata* and *Padina minor*. *D. dichotoma* and *P. minor* are brown seaweed of family Dictyotaceae and Sargassacea respectively. Sargassaceae family is the most common seaweeds' family identified locally in which about 25 species of *Sargassum* were reported throughout Malaysia. Five of *Sargassum* species were found in Blue Lagoon, Port Dickson as reported by Wong and Phang (2004). *L. intricata* is categorised as red algae of family Rhodomelaceae (Phang, 2006).

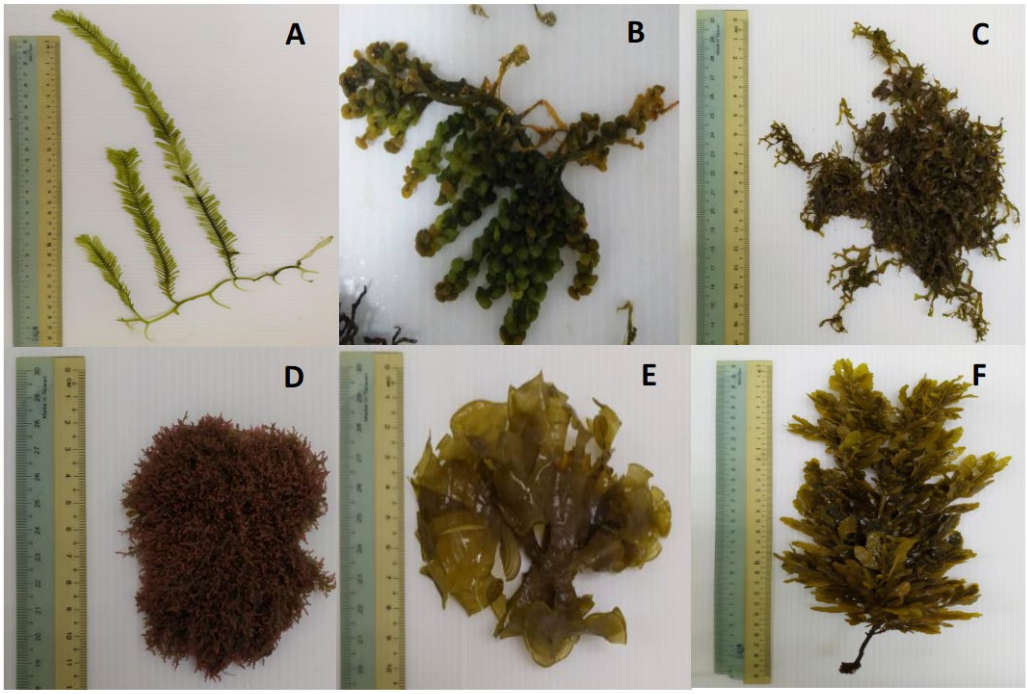

Figure 1: Seaweed species identified based on morphological characteristics. A: *Caulerpa corynephora*, B: *Caulerpa microphysa*, C: *Dictyota dichotoma*, D: *Laurencia intricata*, E: *Padina minor* and F: *Sargassum siliquosum*

Characterization of cellulose in seaweeds

With minor modification, cellulose particles were prepared following the steps suggested by Singh *et al*. (2017). The seaweeds were subjected to grinding, microwave irradiation, bleaching, acid hydrolysis and drying. The dark green and red colour of seaweed becomes paler at the end of the process (Figure 2). Normal distribution of graphs obtained from using Hydro 2000 MU Malvern Mastersizer were

analysed, which indicated the distribution size of cellulose particles ranging from 0.020 to 1300.000 µm. About 0.01% of cellulose particles was detected at 1.529 µm in *C. microphysa* and considered as the smallest one. The largest cellulose particles was detected at 1258.925 µm in *D. dichotoma* and *S. siliquosum* with percentage volume of 1.55% and 0.03% respectively (Table 1).

Figure 2: The end product of *C. corynophera*

Table 1: The biggest and smallest cellulose particles (µm) in all samples

Sample	Smallest size of cellulose particle (µm) The percentage (%)	Biggest size of cellulose particles (µm) The percentage (%)	Range size of cellulose particles (µm) Highest percentage of detection (%)
C. corynephora	2.884 0.04	1096.478 0.20	630.957 to 724.436 13.21
C. microphysa	1.259 0.01	954.993 3.05	630.957 to 724.436 9.70
D. dichotoma	275.423 0.33	1258.925 1.55	630.957 to 724.436 17.23
L. intricata	5.012 0.02	954.993 1.05	416.869 to 478.630 7.38
P. minor	2.884 0.05	954.993 0.75	416.869 to 478.630 8.18
S. siliquosum	2.512 0.05	1258.925 0.03	630.957 to 724.436 14.78

Nanocellulose is a word dealing with nanostructured cellulose, with typical dimension of 3-30 nm in diameter and 100 nm to 1-2 µm in length (Mishra *et al*., 2018). In this study, all cellulose particles isolated was in micro size (Table 1). Mishra *et al*., (2018) classified the cellulose particles with size exceeding 1 µm in diameter and length as microcrystalline cellulose (MCC). Therefore, the isolated cellulose particles from local seaweeds in this study are classified under MCC. Helbert *et al*.

(1996) stated that MCC can be further processed into cellulose nanocrystals, the one with desired nano-sized, through sonication or acid hydrolysis using hydrochloric acid. It is so because MCC is the origin of nanocrystalline cellulose (NCC)

Conclusion

In this study, six local species of seaweeds known as *Caulerpa corynophera*, *Caulerpa microphysa*, *Dictyota dichotoma*, *Laurencia intricata*, *Sargassum siliquosum* and *Padina minor* were identified by means of morphology. Smallest cellulose particles were produced from *C. mycrophysa* but the size was not in nanometer as desired. However, the obtained product can be further processed to get the desired nanocellulose. Thus, this study marks the beginning of wider exploration of local seaweeds for production of nanocellulose.

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