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Isolation and characterization of nanocellulose from water hyacinth, *Eichhornia crassipes* and its textile dye remediation

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Abstract Cellulose isolated from the invasive aquatic weed Eichhornia crassipes (water hyacinth) demonstrated significant potential as a sustainable nanomaterial for environmental applications. A high yield of cellulose (56.68 g per 100 g of dried biomass) was obtained through sequential chemical treatments. Structural and morphological characterization using SEM and TEM revealed the formation of well-defined nanofibrillar structures, while FTIR and XRD analyses confirmed the successful removal of non-cellulosic components and the presence of crystalline cellulose with a crystallinity index of 50.5%. The isolated nanocellulose exhibited excellent dye adsorption capacity, achieving maximum removal efficiencies of 98% for malachite green, 74% for Congo red, 60% for methylene blue, and 32% for phenol red. These findings highlight the effective conversion of water hyacinth into value-added nanocellulose with promising application in textile dye remediation and wastewater treatment.

Keywords: Water hyacinth, Cellulose, Biopolymer, Lignin, Biodegradable, Adsorption

Introduction

Natural fibers are obtained from different sources such as plants, algae, and fungi and these fibers are used as alternative reinforcing agents in biopolymer production. Generally, this natural fiber contains three distinct polymeric components namely cellulose, hemicellulose, and lignin. Hemicellulose is unstructured polymer with high biodegradable, high water absorbing property and it easily breakdown with heat. Cellulose' is the major constituent of numerous plant fibers like cotton, bamboo, jute, sugar cane, hemp and also produced by certain bacteria, and fungi (Asrofi *et al.,* 2018) Cellulose is a crystalline structure of linear polymer unit' of β 1-4 D glucopyranose monomers. Cellulose concentrations vary in higher to lower plants and cellulose-is rich in higher and woody plants. High cellulose content gives stronger mechanical support. The higher concentration of cellulose and its high degree of

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polymerization increase the mechanical and thermal properties of cellulose (Istirokhatun *et al*., 2015).

Eichhornia crassipes commonly called as water hyacinth (WH) is one of the high celluloses containing plant which grows very fast and floats on the upper surface of the water. It originated in South American countries but is available worldwide. It belongs to the family *Pontederiaceae* (Pakutsah and Aht-Ong, 2020). It is an aquatic weed shrub that spreads in water ecosystems and causes environmental issues. WH form a dense growth in water bodies which resist the sunlight, reduce the temperature, change the pH and oxygen levels of water bodies, completely reduce the gas conversation at the water surface, increase the water loss through 'transpiration' spoiling the use of streams (Sundari and Ramesh, 2012).

Naturally, water hyacinth is present with rich fibers and contains 60% cellulose in general, however, it varies depending on the environment. WH is an easily available, low cost, high cellulose containing weed material but it can cause huge negative impact on water bodies and aquatic organisms. The destruction and removal of WH by chemical or mechanical process is a labor intensive. The petiole of water hyacinth contains high fiber content (Wulandari *et al*., 2016) However, due to its high cellulosic nature, we can use this plant weed material for the separation of cellulose polymer for various applications. Hence, the present study is attempted to isolate, characterize cellulose fiber from water hyacinth and its further evaluation for dye absorption properties.

Materials and methods

Collection of Eichhornia crassipes (water hyacinth)

Water hyacinth, *Eichhornia crassipes* plant was collected from the canal water near Murugan temple at Thiruporur (Lat. 12^0 4331'N Long.80^0 1120E), Tamil Nadu, India. The collected plant material was completely washed with water twice. Then the leaf and stem portions are cut into 3-4 cm size pieces and kept under direct sunlight for 3 days for complete drying. The dried plant material was grinded into powder using a Mixer Grinder. The plant powder was stored in a clean polythene bag until further study.

Isolation of cellulose from water hyacinth

Alkali treatment

About 15 Grams of water hyacinth plant powder was taken in a 1000 ml beaker and added with 600 ml of distilled water along with 250 ml of 2% NaOH sodium hydroxide and kept for 6 hours with continuous stirring at 85°C. This

process is done to remove the lignin content which is present in the plant sample. The treated fiber was filtered and rinsed with distilled water until it reaches the neutral pH level. Then the plant residue was dried in direct sunlight for three days (Mochand *et al*., 2018).

Delignification

In the delignification step, already dried raw cellulose material was mixed with acetic acid and hydrochloric acid in a 4:2 ratio with the pH of 5 at 90°C with continuously stirring for 3 hours. In this process the sodium hydroxide helps to degrade the plant cell wall and remove the excess lignin inside the cell whereas acetic acid solvent which helps in the esterification of samples in the residue (Muluneh *et al*., 2019).

Bleaching

The bleaching process makes the fiber brown to white color. This was done to extract the residual lignin completely and this bleaching was done with hydrogen peroxide and acetic acid in a 4:1 ratio with the pH range 5. The mixture was stirred at a with magnetic stirrer at 60°C for 3 hours. During this bleaching process, the water hyacinth fiber completely became white. The bleached fiber was rinsed with distilled water two times to remove the acid and neutralized the pH range until reaches pH 7.

Acid hydrolysis

The acid hydrolysis process aims to increase the crystallinity of the fiber. It helps to obtain a small and unique form of cellulose. The acid hydrolyzed process of water hyacinth was done by adding 5M hydrochloric acid HCl and stirred at 300 rpm at 65°C for 8 hours. After this completion again the water hyacinth fiber was hydrolyzed with 3M hydrochloric acid stirred at 300 rpm at 65°C for another 8 hours. Then the fiber was washed twice with distilled water until reaches the neutral pH.

Sonication

The sonication process helps to homogenize the individual cells in the suspension. This was done by dissolving Briefly, 1 gram of raw cellulose was added into 100 ml of distilled water and kept in an ultrasonic cell crusher at 20 kHz with 300 W for 2 hours or more if needed. The temperature of water hyacinth fiber nanocellulose suspension was maintained at 50°c.

Centrifugation & Freeze drying

The centrifugation process helps to remove excess water content present in the nanocellulose fiber. Two ml of liquid nanocellulose suspension was taken

into centrifuge tubes at 3000 rpm at 40°c for 30 min after completion the excess water was removed from the nanocellulose fiber. The resultant nanofiber was free dried using lyophilizer. The wet weight of the cellulose nanofiber was quantified and expressed in 56.68 /100 grams of dried water hyacinth.

Characterization of nanocellulose

Characteristics of water hyacinth nanofibre was studied by SEM, TEM, FTIR, XRD, particle size and zeta potential analysis at the Advanced characterization facilities at Sathyabama Institute of Science and Technology, Chennai, India. The surface structure of BC was studied by SEM analysis using FESEM (Supra55-Carl Zeiss, Germany). The ultra structure of cellulose nano fiber was studied using Transmission Electron Microscope (JEOL JEM 2100). The FT IR spectrum of BC was determined using the instrument FT/IR-6600 type A model in the region of 500 to 4500 at ambient temperature and the signal obtained at 1 cm^{-1} resolution. The X-ray diffraction (XRD) analysis of BC sample crystallinity index was carried out with a wavelength of 1.540560 Å at the 2θ˚ of 22.80 observation using XRD Smart Lab-9kW-RIKAGU, Japan (Wulandari *et al*., 2016).

Absorption study of nanocellulose

The textile dye absorbing property of nanocellulose was determined by flask method using four different dyes *viz*., congo red, phenol red, methylene blue and malachite green. All the dye solutions were prepared at different concentration ranges from 2.5 – 20 mg/100 ml of distilled water and sterilized by autoclaving. Then 50 mg of nanocellulose was added into all the dye containing flasks.

All the flasks were kept for 30 min in an orbital shaker at 150 rpm. Then the flasks were kept at static condition for 10 min for the settling of cellulose material. Then the supernatant of each dye flask was collected and its adsorption was measured at respective wavelengths i.e. 460 nm for malachite green, 663 nm for methylene blue 663 nm, 498 for Congo red 498, and 423 for phenol reducing in 96 well plate using microplate reader (Biotek). Absorption of untreated dye solution was also taken t respective wavelengths and treated as control (Ramos-Vargas *et al*., 2020).

Percentage of absorbance % = $C_0 - T_t / C_0 \times 100$

Where C_0 is the absorption of the control

Where T_t is the absorbance of the Test

Results

Isolation of cellulose from water hyacinth

Nanocellulose was extracted from Eichhornia crassipes through sequential alkali treatment, delignification, bleaching, acid hydrolysis, and sonication (Figure 1). During alkali treatment with NaOH, removal of hemicellulose and lignin was indicated by a visible change in fiber color from green to yellowish-green. Subsequent bleaching using hydrogen peroxide and acetic acid converted the fibers from brownish-yellow to bright white, confirming effective delignification. Acid hydrolysis with HCl reduced microcrystalline cellulose into smaller crystallites, and sonication further separated these into individualized nanofibrils.

A final yield of 56.68 g of purified nanocellulose was obtained from 100 g of dried biomass.

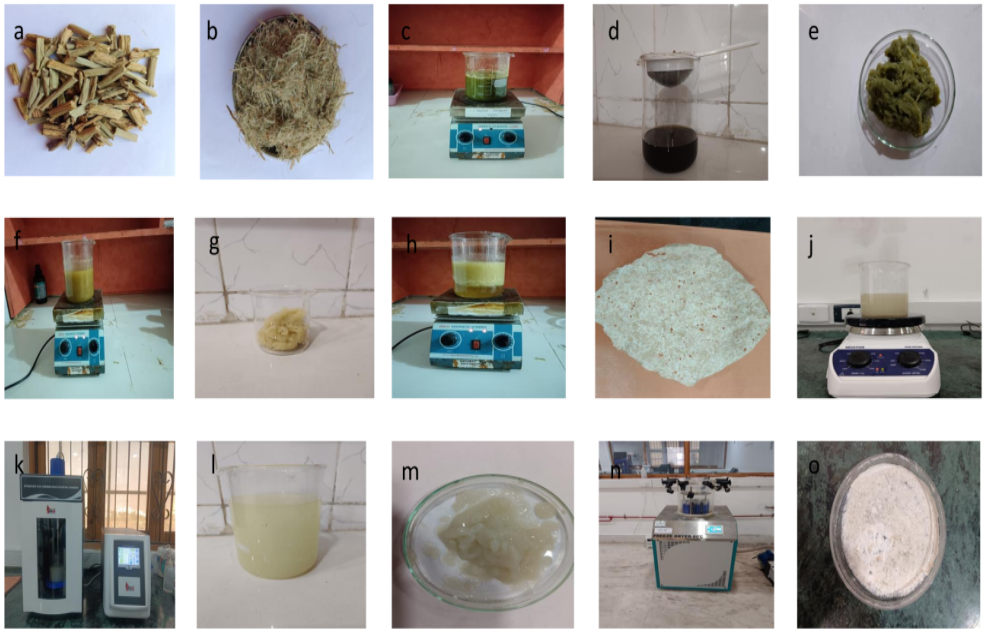

Figure 1. Sequential steps in cellulose extraction from water hyacinth: (a) dried plant, (b) powdered sample, (c) alkali lysis, (d) lignin filtration, (e) lysed fiber, (f) delignification, (g) delignified fiber, (h) bleaching with HCl, (i) dried cellulose fiber, (j) bleaching with H_2O_2 + CH_3COOH, (k) sonication, (l) nanocellulose suspension, (m) centrifuged product, (n) lyophilization, (o) final nanocellulose

Characterization of water hyacinth cellulose

Scanning Electron Microscopy (SEM) and Transmission Electron Microscopy (TEM)

SEM images showed a dense and entangled fibrillar network with smooth surfaces after removal of non-cellulosic components. TEM confirmed nanofibrils in the 50–100 nm range, displaying well-defined crystalline domains. These structural features indicate successful conversion from macro-fibrils to nano-sized fibrils (Figure 2).

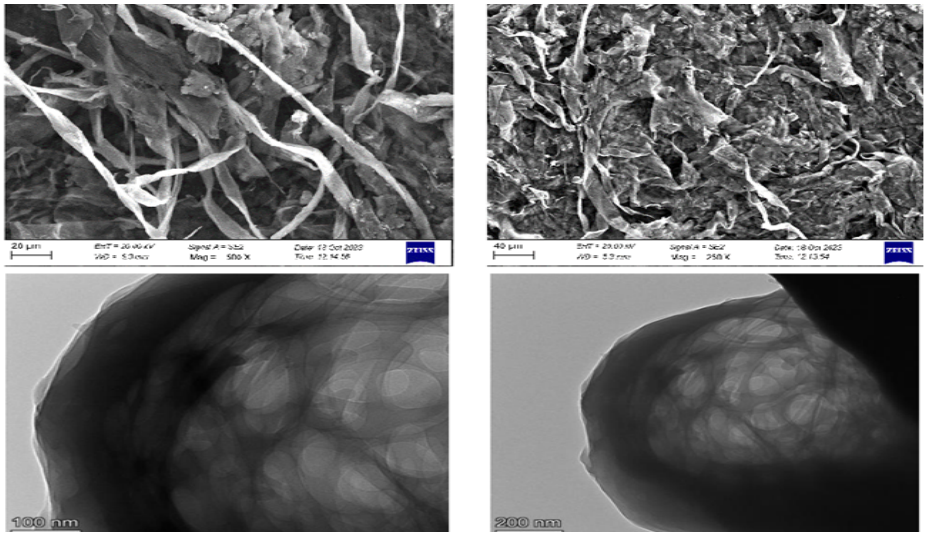

Figure 2. SEM, TEM analysis of WH nanocellulose

Fourier transform infrared (FTIR)

FTIR spectra showed a broad O–H stretch at ~3330 cm^{-1}, C–H stretching at ~2905 cm^{-1}, CH_2 bending at ~1368 cm^{-1}, and C–O–C stretching at ~1027 cm^{-1}, confirming cellulose polysaccharide structures (Figure 3). Absence of lignin peaks (~1510 cm^{-1}) and hemicellulose peaks (~1730 cm^{-1}) indicates efficient purification.

X-ray diffraction (XRD)

XRD analysis (Figure 4) revealed a characteristic cellulose I peak at $2\theta \approx 22°$. The crystallinity index was calculated as 50.5%, confirming a well-organized cellulose structure comparable to other plant-derived nanocelluloses.

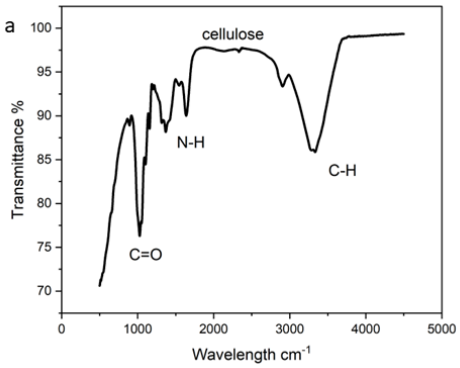

Figure 3. Give separate figure number for FTIR analysis of WH nanocellulose

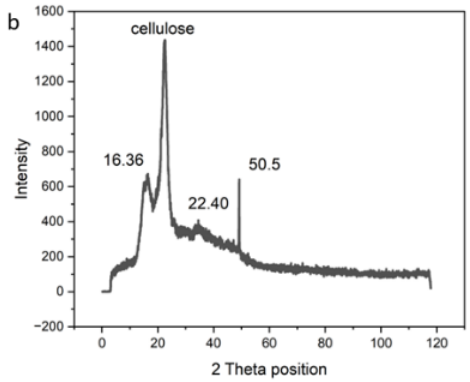

Figure 4. XRD analysis of WH nanocellulose

Dye absorption assay

Nanocellulose exhibited high adsorption capacity for various dyes. Maximum removal efficiencies were:

Equilibrium was reached within 6 hours for all dyes. Increasing adsorbent dosage improved dye removal until saturation (Table 1, Figures 5 and 6).

Table 1. Dye removal efficiency of water hyacinth nanocellulose

Dye	Removal Efficiency
Malachite green	98%
Congo red	74%
Methylene blue	60%
Phenol red	32%

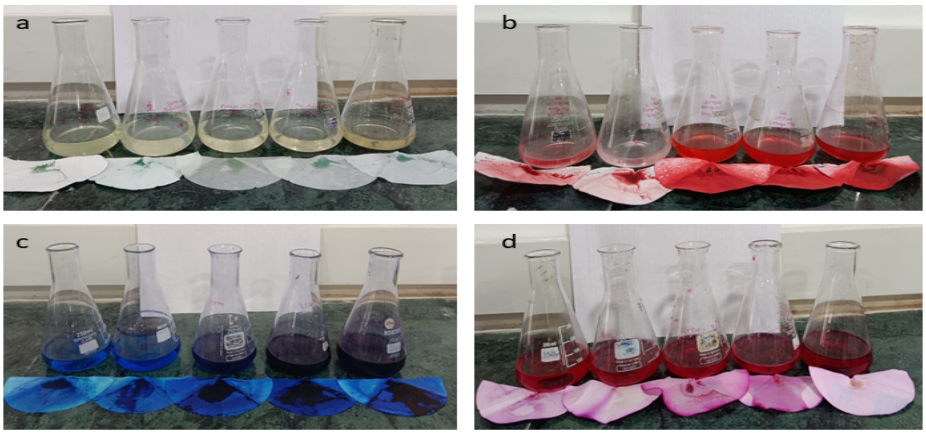

Figure 5. absorption assay of WH nanocellulose a. Malachite green b. Congo red c. Methylene blue d. Phenol red

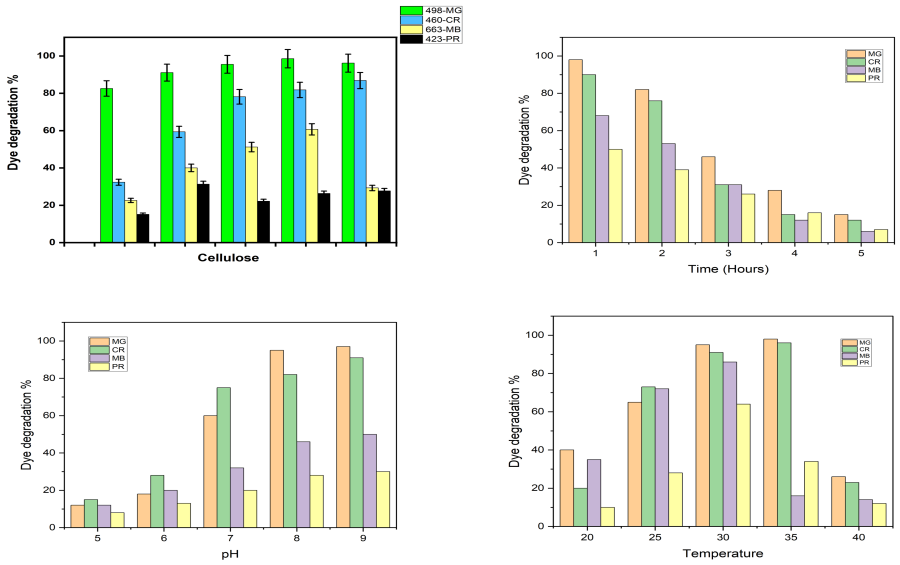

Figure 6. The percentage of dye degradation graph a. Dye absorption b. Time c. pH d. Temperature

Effect of sorbent dosage on adsorption dyes

The effect of nanocellulose dosage on dye removal was evaluated using a sorbent concentration of 20 mg/mL. The dye removal percentage increased steadily with increasing nanocellulose concentration for all four dyes tested. After reaching a maximum removal point, the adsorption percentage became sfi. The highest removal efficiencies observed were 98% for malachite green, 74%

for Congo red, 60% for methylene blue, and 32% for phenol red. The increase in removal efficiency corresponded to the greater availability of adsorption sites at higher nanocellulose dosages.

Discussion

The findings of this study confirm that water hyacinth, a problematic invasive species, can be converted into a high-value nanomaterial through a sustainable waste-to-wealth approach. The sequential treatment process effectively removed non-cellulosic components such as lignin and hemicellulose, as confirmed by the absence of corresponding peaks in FTIR spectra and supported by similar observations in other plant-based nanocellulose studies (Asrofi *et al*., 2018; Sundari and Ramesh, 2012).

The SEM and TEM analyses clearly illustrate the transition from a compact, matrix-bound structure in raw fibers to individualized nanofibrils after processing. This fibrillation increases the specific surface area, a critical factor for enhanced adsorption properties. Such morphological improvements have been linked to better performance in composite reinforcement and pollutant removal applications. (Pakutsah and Aht-Ong, 2020).

The XRD-derived crystallinity index of 50.5% indicates that the extracted nanocellulose possesses a substantial proportion of ordered crystalline domains. This structural feature is known to enhance mechanical strength, thermal stability, and resistance to microbial degradation, making the material suitable for industrial and environmental applications. (Trilokesh and Uppuluri, 2019).

The dye adsorption experiments demonstrated high removal efficiencies, particularly for malachite green and Congo red, suggesting strong interactions between the dye molecules and nanocellulose surfaces. These interactions likely occur through hydrogen bonding, electrostatic attraction, and van der Waals forces, as previously described in cellulose adsorption studies. The differences in removal efficiency between cationic and anionic dyes can be attributed to variations in molecular size, structure, and binding affinity toward cellulose functional groups (Ramos-Vargas *et al*., 2020; Ma *et al*., 2020).

Overall, the results underscore the dual benefits of mitigating water hyacinth proliferation while generating a functional nanomaterial with wide-ranging applications, including wastewater treatment, bio-composite fabrication, and development of environmentally friendly adsorbents. The integration of environmental remediation with high-value product generation aligns well with circular economy principles and offers a scalable pathway for sustainable resource utilization.

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Conflicts of interest

The authors declare no conflict of interest.

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