Preparation of Spherical Nanocellulose by Anaerobic Microbial Consortium

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Abstract — **This work demonstrates the preparation of spherical nanocellulose from microcrystalline cellulose by controlled hydrolysis using anaerobic microbial consortium. The nanocellulose formed during the degradation of microcrystalline cellulose was separated by ultra filtration membrane and purified by differential centrifugation. The purified nanocellulose was characterized by nanoparticle size analyzer, atomic force microscopy and Fourier Transform infrared spectroscopy. While the conventional process of nanocellulose preparation using concentrated sulfuric acid hydrolysis resulted in the formation of nanowhiskers, controlled hydrolysis by anaerobic microbial consortium yielded spherical nanocellulose.**

Keywords- anaerobes; atomic force microscopy; hydrolysis; nanocellulose; ultrafiltration

I. INTRODUCTION

Cellulose, the most abundant biomass on Earth, is a linear homopolymer of β-(1→4) linked D-glucose units. Cellulose is a straight chain polymer with multiple hydrogen bonds between hydroxyl groups of glucose and oxygen molecules on the same or on a neighbor chain, resulting in a high tensile strength. Cellulose is the structural component of primary cell wall of plants, many forms of algae and fungi. For industrial use, cellulose is obtained from wood pulp and cotton. The estimated bending strength and modulus of cellulose nanofibrils were 10 and 150 GPa, respectively [1, 2]. Conventional process for preparation of nanocellulose involves the use of 63.5% (w/w) sulfuric acid on microcrystalline cellulose (MCC) that results in cellulose nanowhiskers with a length between 200 and 400 nm and a width less than 10 nm and a yield of 30 % [3]. The hydrolyzing MCC with sulfuric acid involves esterification of hydroxyl groups to yield acid half-ester or the so-called 'cellulose sulfate' [4]. The presence of sulfate groups on the surface of nanocellulose results in negatively charged surfaces above acidic pH. This anionic stabilization via the repulsion forces of electrical double layers was shown to be very efficient in preventing the aggregation of nanocellulose driven by hydrogen bonding [5]. But, acid hydrolysis process is energy intensive, environmentally hazardous and the surface of nanocellulose is chemically modified by an inorganic element.

To overcome the problem of acid hydrolysis process, our research group has reported a novel microbial process for controlled hydrolysis of MCC to prepare nanocellulose [6]. This process utilized the cellulase enzyme secreted by *Trichoderma reesei* to reduce the size of MCC prepared from cotton in a controlled manner. The nanocellulose produced was whiskers and retained the original chemical characteristics of cellulose. This process requires handling of pure fungus culture and maintaining stringent conditions for culture growth. In this work, we have developed a process for production of spherical nanocellulose by hydrolyzing MCC using anaerobic microbial consortium. This will increase the process efficiency and ease of handling.

II. MATERIALS AND METHODS

The anaerobic microbial consortium being maintained at this institute was enriched with cellulase producers using MCC as sole carbon source. The enriched culture was maintained by sub-culturing once in a span of 30 days. The MCC was prepared from cotton fibres by conventional hydrochloric acid hydrolysis (4N) and the resultant MCC was sieved through various sieves and the size range of 45 – 53 μm was used for further work.

For anaerobic microbial consortium, the basal salt medium with trace element solution was used (Table 1) with MCC as the sole carbon source (1%). Shaking of the medium was very much essential to keep the MCC in suspension. The temperature of incubation was maintained at 35 °C in a shaking water bath.

TABLE I.   MEDIA COMPOSITION FOR ANAEROBIC MICROBIAL CONSORTIUM

Basal Salt Solution		Trace Element Solution	
Chemicals	*Composition (g / L)*	*Chemicals*	*Composition (g / L)*
$NaHCO_3$	8.00	$ZnSO_4 7H_2O$	0.10
KH_2PO_4	1.00	$MnCl_3 4H_2O$	0.03
K_2HPO_4	3.00	H_3BO_3	0.30
$CaCl_2 2H_2O$	0.03	$CoCl_2 6H_2O$	0.20
$MgCl_2 6H_2O$	0.08	$CuCl_2 2H_2O$	0.01
NH_4Cl	0.18	$NiCl_2 6H_2O$	0.02
L-cysteine-HCl	0.17	$NaMoO_4 2H_2O$	0.03
		$FeCl_2 4H_2O$	1.50

The headspace of the anaerobic vials was flushed with anaerobic gas mixture (10% Hydrogen, 10% CO_2 and 80 %

Nitrogen). All the experiments were carried out in duplicate and the mean was analyzed. The incubation was carried out for two periods of time viz., 7 days and 14 days. After incubation, the broth was centrifuged at 1000 rpm to remove cell biomass and analyzed by particle size analyzer, AFM and FTIR.

The particle size distribution was measured using the Nicomp[TM] 380 ZLS size analyzer. Size calibration was carried out using 90 nm size polystyrene latex spheres. The size distribution was obtained based on the dynamic light scattering and autocorrelation principle. The mean diameter of the particles was calculated from their Brownian motion via the Stokes-Einstein equation. For this, HeNe laser (632.8 nm) was used and scattering intensity was analyzed by Avalanche photodiode detector at 90° orientation.

The atomic force microscopic (AFM) analysis was carried out using a diInnova AFM (Veeco, Santa Barbara, CA, US) equipped with 90 µm scanner. A drop of nanocellulose suspension was deposited onto a freshly cleave mica surface and dried under IR lamp. The imaging was carried out in tapping mode in air at room temperature. The silicon nitride cantilever with a spring constant of 40 Nm[-1] was used. The scan rate of 1.0 Hz and 512 lines per 5 um was used for scanning. For FTIR analysis, the freeze dried nanocellulose was diluted with potassium bromide in the ratio of 1:100 and made into a pellet. This pellet was analyzed using an IRPrestige-21® FTIR in transmission mode. For comparison, pristine MCC was used for analysis. The spectra recorded were the average of 64 scans and the contribution of background was accounted for during analysis.

III. RESULTS AND DISCUSSION

The anaerobic microbial consortium enriched with cellulase producers was used to hydrolyze MCC in a shaking water bath. The process of hydrolysis was very slow and it took minimum of seven days to have significant growth of microorganisms. The entire setup was kept in dark to avoid the growth of photosynthetic microorganisms. After seven days of incubation, the broth was centrifuged at 1000 rpm for 15 min to remove the cell biomass and un-hydrolyzed MCC. The supernatant was filtered through 1 um filter and the filtrate was analyzed for particle size, AFM and FTIR. The yield of nanocellulose was calculated by analyzing the cellulose content in the filtrate. The yield of nanocellulose was 6.72 % (± 0.26) after seven days of hydrolysis and 12.30 % (± 0.4) after fourteen days of hydrolysis.

The particle size analysis graph given in figure 1 indicates the presence of bimodal distribution of nanocellulose particles. The size ranges for two peaks after 7 days of incubation were 142.0 ± 25.1 and 796.3 ± 131.4 nm while after 14 days of incubation were 313.0 ± 49.6 nm and 1209 ± 155.8. This bimodal distribution is supported by AFM analysis of nanocellulose. The AFM image shows the predominance of two sized particles in the entire scan range. Also, the particles were very much spherical in contrast to

the whiskers obtained in reference [6]. The AFM image of nanocellulose is given in figure 2.
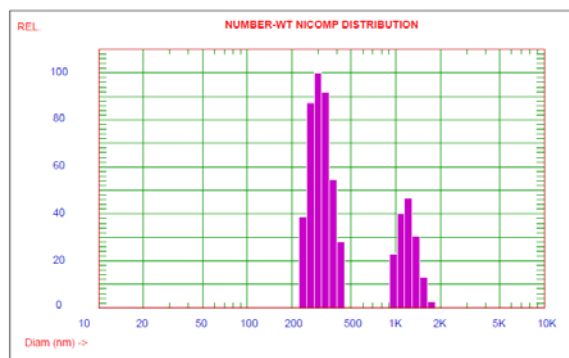

Figure 1.   Particle size distribution of nanocellulose after 14 days of incubation under stirring at 35 °C.
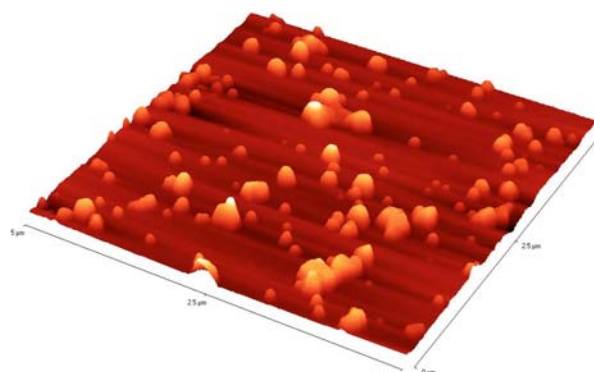

Figure 2.   AFM image of nanocellulose after 14 days of incubation under stirring at 35 °C.

FTIR analysis was carried out to analyze the surface chemistry of nanocellulose. Figure 3 shows the FTIR spectrum of both pristine MCC and nanocellulose and presence of similar peaks indicates the preservation of cellulose structure. The characteristic peaks for cellulose [7] are the hydrogen-bonded stretching at 3344 cm^{-1}, the OH bending of the adsorbed water at 1646 cm^{-1}, the CH stretching at 2900 cm^{-1}, the CH and OCH in-plane bending vibrations at 1432 cm^{-1}, the CH deformation vibration at 1373 cm^{-1}, the COC, CCO, and CCH deformation modes and stretching vibrations in which the motions of the C-5 and C-6 atoms are at 898 cm^{-1}, and the C-OH out-of-plane bending mode around 670 cm^{-1}.
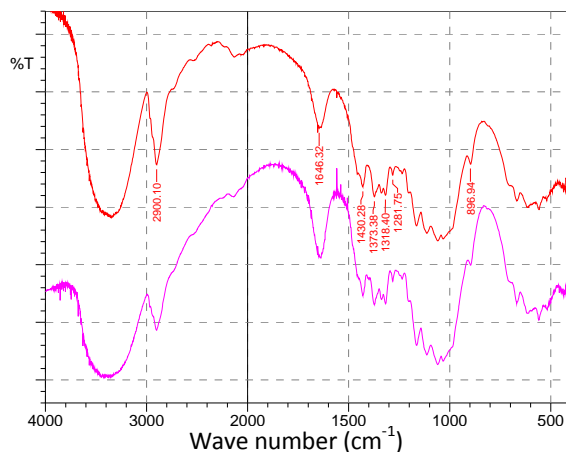
Figure 3.   FTIR spectra of pristine MCC (a) and nanocellulose (b).

Apart from use in bionanocomposites, nanocellulose finds applications in health care like personal hygiene products, biomedicines, cosmetics etc [8]. Nanocellulose, without any surface sulfation, is safe and biocompatible. Conventional sulfuric acid hydrolysis imparts 0.73 % (w/w) of sulfur content in nanocellulose [9] while the microbial hydrolysis retains its original chemical nature.

IV.   CONCLUSION

Microbial preparation is an eco-friendly process to synthesize nanocellulose with the help of enzyme hydrolysis. Enriched anaerobic microbial consortium is efficient in hydrolyzing MCC to nanocellulose in a span of 14 days. The maximum yield achieved was 12% by this process. The advantage of microbial hydrolysis in preserving the chemical structure of cellulose and ease of handling indicates their scope for commercial exploitation.

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