Synthesis of Nano-Cellulose from Okra Fibre and FTIR as Well as Morphological Studies on It

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Abstract: Nano-cellulose was extracted from Okra fiber by various chemical treatment such as alkali treatment, bleaching and then by acid hydrolysis. The final products were characterized by means of Infrared Spectroscopy (FTIR) and Scanning Electron Microscope (SEM). FTIR results showed that the hemicelluloses and lignin were removed from the extracted nano-cellulose. On the other hand, SEM analysis showed that the surface morphology of raw fiber, bleached fiber, alkali treated fiber and nano-cellulosic fiber. The surface of raw fiber was rough, not plain and smooth but the surface of Crystalline Nano Cellulose (CNC) became plain, smooth and not rough for the removing of lignin and fatty and waxy materials from it and simultaneously there is a honey comb structure appeared which will be very helpful for producing nano composites with polymeric materials. Size of the okra fiber was cellulose reduced into nano-sized particles.

Keywords: Nano-Cellulose, Cellulose, Acid Hydrolysis, Natural Fibers, Surface Morphology

1. Introduction

1.1. Okra Plant

Okra plant (*Abelmoschus esculentus*) is of the Malvaceae or mallow family along with cotton, hollyhock, rose or Sharon and hibiscus. It is known by many names such as Lady fingers, Gombo, Okro, Ochro, Okoro, Quimgombo, Bhindi, and Bamieh [1]. A tall-growing, warm-season, annual vegetable, okra has large, attractive, hibiscus like yellow flowers; heart-shaped, lobed leaves, with long stems attached to a thick woody stem. The fruit, a long generally ribbed fuzzy pod developing in the leaf axil, grows rapidly after flowering. The edible part is the fruit pod, which varies in color from yellow to red to green. The upright plant averages between 3-6 feet or more with varieties for both temperate and tropical areas. Okra plant is grown abundantly in Bangladesh. At present, these are waste after collecting fruits. The fibers are obtained mainly from the stem of the plant. About 37 kg (average weight) of stem yields about 2 kg of good quality fiber; the yield is 2-2.5% of dry fibre. It

can be extracted by hand scraping, by retting, or by using raspador machines; it can also be extracted chemically, for example by boiling in NaOH solution. Extraction of the fibre for local use (in cordage) or for cottage industries is due to high cellulose and low lignin content, its use in the paper industry (tissue, filters, specialty nonwoven, document, printing, surgical and hygienic applications, coffee bags, meat casings, etc.) have been reported. Unspecified parts of the plant were reported in 1898 to possess diuretic properties; this is cited (or simply stated) in many sources associated with herbal and traditional medicine. Okra (and rhubarb, beets, spinach, Swiss chard, sweet potatoes, tea, chocolate and soy products) is rich in oxalates; the Mayo clinic recommends that people who tend to form calcium oxalate kidney stones may benefit from restricting such foods [2].

Cellulose, the most abundant polymer on earth, widely distributed over a variety of sources i.e., marine animals, plants and bacterial sources, being a fibrous, tough, linear syndiotactic homopolymer is composed of D-anhydroglucopyranose units which are connected by β -(1, 4)-glycosidic bonds [3]. With the growth of the environmental

awareness and consequent upon a series of new policy, the industries are being enforced to investigate for eco-friendly materials to substitute the existing fossil fuel based polymeric materials, in order to help preserve and protect the environment [4]. Due to biodegradability and biocompatibility nano-cellulose has a great interest in the field of biocomposites and biomedicine. Nano-cellulose, the nano-structured form is cellulose nanofibers, nano-crystalline cellulose or bacterial Nano-cellulose produce by the interaction of bacteria [5] Consisting of polymeric matrix and nanosized particles, nanocomposites have been prepared using biobased materials as a matrix and reinforcement for the reduction of the dependence on non-degradable products and moving to a sustainable materials [6]. Cellulose is mainly used to produce paperboard and paper. Smaller quantities are converted into a wide variety of derivative products such as cellophane and rayon [7]. Conversion of cellulose from energy crops into biofuels such as cellulosic ethanol is under investigation as an alternative fuel source [8]. Cellulose for

industrial use is mainly obtained from wood pulp and cotton [9]. Some animals, particularly ruminants and termites, can digest cellulose with the help of symbiotic micro-organisms that live in their guts, such as *Trichonympha*. Humans can digest cellulose to some extent [10] however it mainly acts as a hydrophilic bulking agent for feces and is often referred to as a "dietary fiber".

1.2. Structure and Properties

Cellulose has no taste, is odorless, is hydrophilic with the contact angle of 20–30 [11] is insoluble in water and most organic solvents, is chiral and is biodegradable. Many properties of cellulose depend on its chain length or degree of polymerization, the number of glucose units that make up one polymer molecule. Cellulose from wood pulp has typical chain lengths between 300 and 1700 units; cotton and other plant fibers as well as bacterial cellulose have chain lengths ranging from 800 to 10,000 units [12]

Figure 1. Structure of Cellulose.

1.3. Hemicellulose

Hemicelluloses are polysaccharides in plant cell walls that have beta-(1→4)-linked backbones with an equatorial configuration. Hemicelluloses include xyloglucans, xylans, mannans and glucomannans, and beta-(1→3,1→4)-glucans [13]. These polysaccharides contain many different sugar monomers. In contrast, cellulose contains only anhydrous glucose. For instance, besides glucose, sugar monomers in hemicellulose can include xylose, mannose, galactose, rhamnose, and arabinose. Hemicelluloses contain most of the D-pentose sugars, and occasionally small amounts of L-sugars as well [14]. Xylose is in most cases the sugar monomer present in the largest amount, although in softwoods mannose can be the most abundant sugar. Not only

regular sugars can be found in hemicellulose, but also their acidified form, for instance glucuronic acid and galacturonic acid can be present.

Figure 2. Hemi-cellulose.

1.4. Lignin

Lignin or lignen is a complex polymer of aromatic alcohols known as mono-lignols. It is most commonly derived from wood, and is an integral part of the secondary cell walls of plants [15] and some algae [16]. The term was introduced in 1819 by de Candolle and is derived from the Latin word lignum [17] meaning wood. It is one of the most abundant

organic polymers on Earth, exceeded only by cellulose. Lignin constitutes 30% of non-fossil organic carbon [18] and a quarter to a third of the dry mass of wood. As a biopolymer, lignin is unusual because of its heterogeneity and lack of a defined primary structure. Its most commonly noted function is the support through strengthening of wood (xylem cells) in vascular plants. [19-21].

Table 1. Composition of different types of fibers.

Fiber	Density (gm/cc)	Cellulose %	Hemi Cellulose %	Lignin %
Jute (<i>Corchorus Capsularis</i>)	1.50	55-60	13	8-13
Okra (<i>Abelmoschus Esculentus</i>)	1.25	45-57	21.5	8-12
Agave Fiber (<i>Agave Atrovirens</i>)	1.48	60-68	20	7-10
Palque Fiber (<i>Agave Americanas</i>)	1.45	68-70	15-17	6-7

Figure 3. Structure of Lignin.

In the research presented below, a refinement of conventional FTIR spectroscopy and SEM were used to characterize cellulosic plant fibres.

2. Experimental

2.1. Materials

Okra bast fibre was the raw material for the nanocellulose which was collected from Kushtia near Islamic University region, 10% soap solution was collected from local market, Sodium Hydroxide (NaOH) purity 98% manufacturer BDH (England), Sodium Chlorite (NaClO₂) purity 80% manufacturer BDH (England), Sodium acetate (CH₃COONa) purity 98% manufacturer BDH (England), Glacial acetic acid (CH₃COOH) purity 100% manufacturer BDH (England), Sodium meta-bi-sulphite (Na₂S₂O₅) purity 99.9% manufacturer E. Marck Ltd. Germany, Sulfuric acid (H₂SO₄) purity 98% manufacturer BDH (England).

2.2. Methods

2.2.1. Collection and Preparation of Okra Fibre

The okra (*Abelmoschus esculentus*) fibre of different species is found in the various regions in Bangladesh. For investigation, the okra fiber was collected from Kushtia. The fibre was collected from okra plant, when plants get mature. The plants when mature may attain a height 5 feet. It was cut down and tied up in bundles. The plant was then retted into dirty water for 13 to 15 days; the fibre was then separated from cementing and gummy materials. The fibre was washed in clean water for several times and dried in air without exposing sunlight. Finally, the fibre was dried in an electric oven at 105°C and stored in a desicator [22].

Figure 4. Raw okra fibre.

2.2.2. Scouring of Okra Bast Fibre

The removal of impurities such as dirty materials, fatty, waxy and gummy substances from textile materials is called scouring [23]. It was carried out by the use of surface-active agents, such as soda and detergents. About 30 cm from the bottom of the fibre was discarded and it was then cut into three equal parts (20 cm) viz. the top, the middle and the bottom. Middle portion of the fiber was used for investigation. Okra bast fibre was scoured in a solution containing 5 mg Na₂CO₃ and 5 gm detergent per liter of water in a large beaker. The ratio of the fiber to solution was 1:50. The solution with okra fibre was heated at 60°C for 30 min. Then the fiber was thoroughly washed with distilled water for several times. Finally, it is dried at (100-105)°C in an electric oven and stored in a desicator.

2.2.3. Alkali Treatment

The study used okra fibres as a test material. Alkali treatment was applied to the okra fibre surface to improve its mechanical properties [24]. Treatment was carried out by dipping small bunches of fibres 17.5% concentrated sodium hydroxide solutions. The treatment times were 2 hours with constant shaking. Here the temperature should be controlled about 40°C. Such different concentrations of solutions elucidated effects of alkali concentration on the mechanical properties of okra fibre. After alkali treatment the fibres were washed for a few minutes with distil water. Then the filtration operation was completed by filter cloth. The residue was finally called Alpha cellulose and filtrate solution contain hemi-cellulose and lignin. Then this Alpha cellulose was sent for bleaching operation.

Figure 5. Scoured okra fibre.

2.2.4. Bleaching

For the purpose of bleaching okra fiber was dried at 90°C and was treated with 0.2% or 2 gm per liter sodium chlorite (NaClO_2) solution was dissolved in a known volume of water and its pH was 10.6, lower to 4 by the acetic acid. A buffer mixture of pH 4 was prepared and added to the chlorite solution in the proportion of 1 ml of buffer solution for every 10 ml of chlorite solution, to ensure that pH remained at 4 throughout the progress of the reaction. The bleaching process was carried out by the digesting fiber for about 2hr. After bleaching, fiber was washed with distilled water. Then fiber was washed and dried in open air and then stored in a desecrator. This operation was carried out again at same condition. This is called double bleaching of fiber. The fibre was again washed with distilled water and dried and stored in a desecrator.

Figure 6. Bleached *Abelmoschus esculentues* (Okra fibre).

2.2.5. Nano-Cellulose

At first the bleached okra cellulose was cut down into as small as possible size. Then, cellulose nanofibers were prepared by the double-acid hydrolysis of okra fiber. The hydrolysis was carried out with H_2SO_4 solution (60 wt. %) at 40°C under continuous stirring with magnetic stirrer on an electric hot plate. The fiber to liquor ratio was 1:10 (10 ml of sulphuric acid for 1 g of cellulose). After 30 minutes, the hydrolysis was stopped by adding 5 fold excess distilled water or ice cool water (50 ml) to the reaction mixture. Again start stirring about 30 minutes. Then the resulting mixture was cooled down at room temperature and centrifuged. The solid fraction was washed off by distilled water until to obtain a neutral pH. Finally, the Nano-cellulose suspension was obtained by stirring the solid fraction with required amount of water. The newly generated suspension was dispersed into “ethanol or absolute alcohol” into a safe as well as clean glass vessel.

The photographic representation is given below: Nano cellulose dispersed into the ethanol or absolute alcohol.

Figure 7. Nano-cellulose from okra fiber.

3. Result and Discussion

3.1. Fourier Transform Infrared Radiation Analysis

FTIR spectroscopy is generally carried out to investigate intermolecular and intramolecular hydrogen bonding [25] of raw okra fiber, alkali treated fiber (α -Cellulose), bleached fiber and nano-cellulosic fiber because FTIR is the most frequent technique to study inter-molecular and intra-molecular interaction in polymer. Prior to characterization by FTIR, raw okra fiber, alkali treated fiber (α -Cellulose), bleached fiber and nano-cellulosic fiber were washed according to the indicated procedure. The Fourier transform infrared spectroscopy (FTIR) spectra of samples were taken on Shimadzu IR Prestige-21 spectrometer. Samples were taken in KBr to make a transparent pellet. For each sample five scans were taken at a resolution of 4 cm^{-1} .

Figure 8 represents the FTIR spectra of raw okra fiber. It showed the characteristic broad absorption of the raw fibre found at 3903.63-3728.40 cm^{-1} due to the free -OH group in polymeric association [26] and 3354.21-3321.12 cm^{-1} due to hydrogen bonded -OH stretching vibration (ν_{OH}) of the cellulose structure of raw okra fiber. The peak at 2895.15-

2821.06 cm^{-1} was assigned to the C-H stretching vibration (ν_{CH}) from the $-\text{CH}_2$ group of cellulose and peak at 1421.54 cm^{-1} and 1226.73 cm^{-1} due to the anti-symmetric bridge C-O-C stretching vibration ($\nu_{\text{C-O-C}}$) of pyranose ring between cellulose and hemicellulose of raw okra fiber (The

peaks centered on 1456 cm^{-1} and 1240 cm^{-1} indicate the existence of lignin and hemicellulose structures, respectively owing to asymmetric C-H deformation of in methyl and methylene groups) [27].

Figure 8. FTIR analysis of raw okra fibre.

The presence of an absorption band near 1654.92 cm^{-1} in the raw banana rachis fibres IR spectra was due to $>\text{C}=\text{O}$ stretching at the carboxylic acid or ester groups in cellulose as shown in figure 8. Lignin showed spectrum in 1500.62 region due to the stretching modes of the benzene ring. The absorption band at 1381.93 cm^{-1} was related to (C-O) stretching 2° alcohols. The strongest bands across spectra at 1103.85 and 1033.85 cm^{-1} were assigned to $-\text{CO}$ stretching. Other bands at 898.83 cm^{-1} originated from $-\text{OH}$ out of plane bending vibrations. This indicates that the compound is fiber. (All peaks are shown in table 2).

In the FTIR spectra of pure alkali treated fiber (in figure 9) the broad absorption band between 3371.57 was related to the $-\text{OH}$ stretching vibrations. The narrow peak recorded at 3246.20 cm^{-1} might be attributed to the $-\text{OH}$ vibrations due to the intramolecular hydrogen bonding. The peak between

2895.15-2821.06 cm^{-1} was assigned to the C-H stretching vibration (ν_{CH}) from the $-\text{CH}_2$ group of α -Cellulose. The presence of an absorption band near 1643.35 cm^{-1} in the alkali treated α -Cellulosic fibre IR spectra was due to $>\text{C}=\text{O}$ stretching at the carboxylic acid or ester groups as shown in figure 9. Lignin showed spectrum in 1597.06 cm^{-1} region due to the stretching modes of special types of unsaturation in the benzene ring [28]. The absorption band at 1381.93 cm^{-1} was related to (C-O) stretching 2° alcohols. The strongest bands across spectra at 1103.85 and 1033.85 cm^{-1} were assigned to $-\text{CO}$ stretching. (All peaks are shown in table 2).

Noteworthy that the absorption bands at 3903.63-3728.40 cm^{-1} due to the free $-\text{OH}$ cm^{-1} , 1421.54 cm^{-1} and 1226.73 cm^{-1} due to the anti-symmetric bridge C-O-C stretching vibration ($\nu_{\text{C-O-C}}$) of pyranose ring between cellulose and hemicellulose as well as at 898.83 cm^{-1} due to $-\text{OH}$ out of

plane bending vibrations were totally absent in pure alkali treated okra fiber (shown in figure 9) because after 17.5%

NaOH treatment there were only α -Cellulose and lignin exist in the fiber.

Figure 9. FTIR analysis of alkali treated okra fibre.

Figure 10 represents the FTIR spectra of bleached okra fiber. Here, the absorption at 3747.69 cm^{-1} for the free -OH group in polymeric association was come back and IR spectrum showed the broad absorption band between 3371.57 cm^{-1} was related to the -OH stretching vibrations. The narrow peak recorded at 3246.20 cm^{-1} might be attributed to the -OH vibrations due to the intramolecular hydrogen bonding. The peak at 2908.65 cm^{-1} was assigned to the C-H stretching vibration (ν_{CH}) from the -CH_2 group of α -Cellulose. At the point of 2154.49 cm^{-1} a new peak was found which is responsible for $\text{C}\equiv\text{C}$ stretching vibration. The presence of an

absorption band near 1651.07 cm^{-1} in the bleached okra fibres IR spectra due to >C=O stretching at the carboxylic acid or ester groups as shown in figure 10. At 1554.63 cm^{-1} an absorption band was appeared due to the stretching modes of special types of unsaturation in the benzene ring.

The absorption band at 1381.93 cm^{-1} was related to (C-O) stretching 2° alcohols. The strongest bands across spectra at 1103.85 and 1033.83 cm^{-1} were assigned to -CO stretching. A peak at 898.83 cm^{-1} originated from -OH out of plane bending vibrations also appeared. (All peaks are shown in table 2).

Figure 10. FTIR analysis of bleached okra fibre.

On the other hand, there is no peak appeared at 1400 cm^{-1} - 1500 cm^{-1} because of the absence of lignin.

And finally, in the FTIR spectra of pure CNC in figure 11 a peak was appeared at 3714.90 cm^{-1} for the sake of free -OH group in polymeric association. Besides this, also a broad absorption band exist between 3471.87 and 3439.03 cm^{-1} was related to the -OH stretching vibrations. The narrow peak recorded at 3377.36 cm^{-1} might be attributed to the -OH vibrations due to the intramolecular hydrogen bonding. The

absorption band at 2893.22 cm^{-1} and $1500\text{--}1250\text{ cm}^{-1}$ originated from the CH and -CH_2 stretching and bending vibrations, respectively. Two absorption bands was found between 2129.41 and 2087.69 cm^{-1} in CNC spectra because of $\text{C}\equiv\text{C}$ stretching vibration. The presence of an absorption band near 1653.00 cm^{-1} in the nano-cellulosic fibres spectra due to $>\text{C}=\text{O}$ stretching at the carboxylic acid or ester groups as shown in figure 11.

Figure 11. FTIR analysis of nano-cellulosic okra fibre.

At 1556.55 cm^{-1} an absorption band was appeared due to the stretching modes of special types of unsaturation in the benzene ring. The absorption band at 1381.93 cm^{-1} was related to (C-O) stretching 2^0 alcohols. The strongest bands

across spectra at 1103.85 and 1033.83 cm^{-1} were assigned to -CO stretching. A peak at 898.83 cm^{-1} originated from -OH out of plane bending vibrations also appeared. (All peaks are shown in table 2).

Table 2. Functional groups with peak of raw, alkali treated, bleached and nano cellulosic okra fiber.

Accounted bond for the absorption	Raw okra fiber (Peak) cm^{-1}	Alkali treated fiber (Peak) cm^{-1}	Bleached fiber (Peak) cm^{-1}	Nano cellulosic fiber (Peak) cm^{-1}
Free -OH group	3903.63-3728.40		3747.69	3714.90
-OH stretching	3354.21-3321.12	3371.57-3246.20	3435.22-3390.86	3471.87-3377.36
C-H str (In alkanes)	2895.15-2821.06	2895.15-2821.06	2908.65	2893.22
$\text{C}\equiv\text{C}$ stretching			2154.49	2129.41-2087.69
-CO str (in ester)	1654.92	1643.35	1651.07	1653.00
C-C str (In benzene)	1500.62	1597.05	1554.63	1556.55
-C-O-C str	1421.54			
2^0 alcohols (C-O) str	1226.73	1392.61	1382.96	1381.03
1^0 alcohols (C-O) str	1103-1033.85	1101.35-1033.85	1163.08-1039.83	1159.22-1029.88
-OH out of plane	898.83		898.83	898.83

3.2. Scanning Electron Microscope

The morphology of samples was observed by scanning electron microscopy (SEM) [29]. The surface morphology of raw okra fiber, alkali treated fiber (α -Cellulose), bleached fiber and nano-cellulosic fiber were observed by scanning electron microscope (FEI QUANTA 200 3D) with an accelerating voltage 10 KV. The surface was coated with 3 nm thick gold before analysis. The micrographs were taken at a magnification of 100 and 250X. All figures of scanning electron microscopy (SEM) i.e raw, alkali treated with 17.5% NaOH, bleached (by 0.4 NaClO₂) and nano-cellulose of okra fibers were shown below in figure 12-15. The SEM micrograph of raw fiber (Figure 12) clearly demonstrated the presence of longitudinally oriented unit cells with more or less parallel orientations. The intercellular space is filled up by the binder lignin and fatty substances, which hold the unit cells firmly in a fiber.

Figure 12. SEM micrograph of raw okra fibers at a magnification of 100X.

In figure 13 showed the comparative electron micrograph of raw and 17.5% NaOH treated rachis fiber. The multi-cellular nature of fiber filament is more clearly revealed in alkali treated fiber.

Figure 13. SEM micrograph of alkali treated okra fibers at a magnification of 250X.

Compared to the raw rachis fiber with 17.5% NaOH treated fiber, it seems that fiber surface roughen on alkali treatment. Evidence of surface roughness and in addition, porosity a on the fiber surface was found in alkali treated fiber. NaOH treatment of cellulose fiber leads to the irreversible mercerization effect, which increases the amount of amorphous cellulose at the expense of crystalline cellulose. The important modification occurring here is the removal of hydrogen bonding in the network structure. The cellulose-sodium hydroxide reaction is thought to be as follows [30]

Alkali treatment improves adhesion, characteristics by removing surface impurities, thus exposing micro-fibrils, which then render the fiber topography with a rough texture.

In figure 14 showed the comparative SEM picture of bleached banana rachis fiber. From this figure the drastic difference in the surface morphology between raw and bleached fiber can be easily visualized. Generally, natural cellulose fiber are negatively charged due to the presence of carboxyl and hydroxyl groups as mentioned in the literature. In case of raw fiber, these groups are covered by lignin, present in the primary wall of the fiber shown in figure 14 where a resinous second phase can be seen on the fiber surface, while the surfaces of bleached fiber which appear more smooth than figure 13. Unlike the raw fibers the surface of bleached fibers seems to be free from surface debris and overgrowth. This is undoubtedly due to removal of surface impurities as well as lignin and hemi-cellulose. The microgram of bleached okra fiber is given below:

Figure 14. SEM micrograph of bleached okra fibers at a magnification of 250X.

And finally, the CNC appeared in figure 15, micro and nanoparticle-like structure, and it is also possible to see some nano-fibrils of CNC particle surfaces although it is not clearly detectable due to higher magnifications ($\times 250$)m. It can be seen that the Nano-cellulose fibers have the diameter at a range of around 400 -800 nm with some micro-cellulose

of several micrometer.

Figure 15. SEM microgram of nanocellulosic okra fibers at a magnification of 250X.

Noteworthy that in the microgram of CNC there is a lot of honey comb structures appeared which is very effective for producing any types of polymeric nano composites. Because, this structures of nano-cellulosic fibers help to interlock the filler materials when they combined with each other. So, a very strong bonding is possible which can provide a strength composite.

4. Conclusions

Okra fibre is a waste product of agricultural sector in Bangladesh. After harvesting the fruit, the plant is cut down and thrown away. Every year a colossal amount of okra plant remain as leftover creating environmental pollution but it can be utilized as a source of cellulose for producing nano-cellulose. Cellulose was extracted from above fibre by an effective two-step process: the removal of hemicellulose and lignin delignification by 0.2% NaClO₂. Then the isolation of nano-cellulose was carried out by 60% of H₂SO₄. Modified fibre contains mostly cellulosic units whereas the raw okra fibre contains cellulose, lignin, and hemicellulose. It has been revealed that due to the bleaching treatment of raw fibre, a substantial portion of lignin, wax and others impurities are removed from the fibre, resulting in the disappearance of the carbonyl group peak. From these results, it can be concluded that the ester groups present in hemicellulose and lignin are more easily removed by an alkali and bleaching treatments respectively. The structural changes were measured by FTIR analysis. Resulting data represents nano-cellulose show higher crystallinity and lower stability as compared to native cellulose. The surface morphology of the nano-cellulose was characterized by SEM analysis. Images disclose the clear arrangement of nano-cellulose. The surface has honey comb structure by which the nano-cellulose is able to produce a very strong composite with polymeric materials. This composite have a tensile strength more than eight times to steel [31].

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Declaration

All the authors do not have any possible conflicts of interest.

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