Cellulose from Cladophorales Green Algae: From Environmental Problem to High-Tech Composite Materials

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ABSTRACT: This article represents the first attempt to summarize the knowledge about the structure and functional properties of green algae cellulose since 1885, when cellulose of algal origin was first described. Here, I systematically discuss the unique structural characteristics of *Cladophora* cellulose. This specific interest in *Cladophora* filamentous green algae is justified by unwanted global socio-environmental problems associated with seasonal blooms by these algae. Thus, the physicochemical properties and the use of this unique material are discussed in a broad context. Several high-tech applications wherein the use of *Cladophora* cellulose is highly beneficial are highlighted. © 2010 Wiley Periodicals, Inc. J Appl Polym Sci 119: 2449–2460, 2011

Key words: biofibers; biopolymers; composites; high performance polymers; nanofibers

WHY CLADOPHORA ALGAE? ENVIRONMENTAL AND SOCIOECONOMIC CONTEXT

The severe eutrophication of coastal areas is one of the biggest environmental threats of our time, as excessive blooming by green algae, triggered by industrial pollutants in water, jeopardizes the fragile balance of ecosystems.[1–3] The increased sliming of shore areas, piers, and fishing equipment is an obvious sign of worsening seawater quality. This sliming is caused by the dense growth of microalgae and filamentous green algae. Such growth also reduces the transparency of the water; this means that the species that grow deeper in the water suffer from a lack of sunlight. Annual filamentous green *Cladophora* algae, which grow on submerged rocks and stones, can then proliferate. Figure 1 shows *Cladophora* algae growing on a rocky substrate. As these algae age and die, they drift into the water, forming dense mats and harming other forms of aquatic life. These mats of filamentous algae eventually fall to the seabed, where they are decomposed by bacteria. This process can consume a considerable amount of oxygen, and the supply of oxygen on the seabed may even run out altogether. The excessive supply of nutrients and consequent eutrophication and oxygen depletion on the seabed all weaken the functioning of marine ecosystems.

Cladophora algae blooms are seasonal and sensitive to changes in light availability.[4,5] However, *Cladophora* algae rapidly adapt to changes in the temperature of the surrounding environment, and therefore, their photosynthesis and respiration rates are largely unaffected by such fluctuations.[6] *Cladophora* green algae prefer large solid substrates with a high roughness and porosity for proliferation.[7] For instance, the probability of *Cladophora* algae eutrophication is higher in areas with large stones with rugged surfaces, for example, on the concrete blocks of peers, whereas small stones with smooth surfaces are less attractive. It appears, however, that the chemical composition of the rocks, for example, sandstone, obsidian, schist, graywacke, pumice, gypsum, limestone, serpentine, or phosphorite, is of minor importance for the eutrophication of *Cladophora* algae.[7]

A clear link between the blooms and high phosphorus and nitrogen levels in the water has been established and ascribed mainly to human activities, such as lawn fertilization, poor maintenance of septic systems, inadequate sewage treatment, agricultural runoff, and excessive use of detergents containing phosphorus.[8,9] When the phosphor content in water is below 1 mg/L, only moderate *Cladophora* eutrophication is observed.[10] Among other factors contributing to algal blooms, the invasion of dreissenid mussels (zebra, quagga, etc) in, for example, the Great Lakes area has been found responsible for increases in algae in regions where phosphorus levels were previously curtailed.[11] This suggested that the increased release of phosphor from the metabolic wastes and feces of dreissenid mussels and the increased water clarity contributed to the new wave of *Cladophora* eutrophication in Lake Eire.[11,12]

Figure 1 Blooming *Cladophora* green algae in the Baltic Sea. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

The excessive blooming of *Cladophora* algae is a global problem, and alarming reports from many regions, including the United States (especially the Great Lakes area), Indonesia, the Philippines, Australia, Japan, and the Baltic region, are common.[13] Because *Cladophora* algae proliferate in shallow waters, it is public beaches and private lawns that suffer first. A thick dense biomass of deteriorating algae washes onto the shores, spreading a pungent septic odor around. Unlike blue-green algae that produce toxins, filamentous green algae as such do not pose a direct risk to human health. However, the rotting algal biomass provides adequate conditions for bacterial growth, especially *Escherichia coli, Salmonella, Shigella, Campylobacter,* and enterococci fecal bacteria.[14,15]

The cleaning of the shores suffering from eutrophication constitutes a heavy burden for local communities because volunteer work is not usually enough to address the problem. Repeated blooming over several years may result in a steady decline in the number of tourists visiting camping sites at small local communities that did not suffer from eutrophication in the past. Dropping prices for real estate are another adverse effect of eutrophication in coastal areas. It is, thus, clear that the detrimental effects of excessive eutrophication by *Cladophora* are crucial not only from a purely ecological perspective but also in the socioeconomic context.[12]

There is a big social demand to determine an industrial utilization for the blooming filamentous algae. A special European Union-sponsored project (LIFE96ENV/S/380) was conducted in Sweden to elucidate the socioeconomic profitability and technical feasibility of the removal of algae.[16] Although it was proven that the removal of algal mats restores the balance of ecosystems—as reflected, for example, by an increased recruitment of fish—there still remains the question of what to do with the

removed algae.[17] Despite the negativism related to the adverse ecological effects of *Cladophora* algae blooms, the latter could be a valuable source of industrially useful materials. The aim of this article is to provide an overview of some of the unique properties of cellulose powder derived from the environmentally polluting green filamentous *Cladophora* algae and discuss some of the applications in which these unique properties would be highly beneficial.

STRUCTURAL CHARACTERISTICS OF *CLADOPHORA* ALGAE CELLULOSE

The insoluble residue left after the removal of alginic acid from seaweeds is called *algic cellulose* and was first described by Stanford in 1885.[18] Similar to native cellulose, it shows color when it is reacted with iodine and sulfuric acid[19] and is soluble in cuprammonium hydroxide and produces acetate.[20] The main question, however, posed in the early 1920s and 1930s was whether the building units of algic cellulose were solely d-glucose connected through (1→4) β linkages or whether substantial amounts of other sugars, such as mannose and galactose, were present. Findings by Percival and Ross[21,22] confirmed that algic cellulose consists solely of d-glucose monomers and shows characteristic X-ray diffraction (XRD) pattern similar to that of native cellulose from land plants.

Regardless of its source, native cellulose (or cellulose I) is a mixture of two allomorphs: cellulose Iα and cellulose Iβ. The triclinic Iα allomorph is predominant in algal–bacterial celluloses, whereas the cotton–ramie types of cellulose are rich in the Iβ allomorph.[23] The cellulose Iα allomorph is more susceptible to enzymatic degradation.[24,25] However, cotton–ramie types of cellulose, which are rich in the Iβ allomorph of cellulose, also contain substantial

amounts of disordered regions, which in turn, are highly susceptible to chemical reactions. Therefore, the overall reactivity of the cellulose substrate will be primarily dominated by the disordered fraction and only secondarily dominated by the relative reactivity of the Iα and Iβ allomorphs.

According to Nicolai and Preston,[26] three groups of algae species can be classified according to their cell wall constituents. Group 1 includes green algae in which native cellulose is the major component of the cell walls and which is usually highly crystalline. To these algae belong those of the Cladophorales (*Cladophora, Chaetomorpha, Rhizoclonium,* and *Microdyction*) and a few members of Siphonocladales (*Valonia, Dictyosphaeria, Siphonocladus,* and *Boergesenia*) orders. Group 2 constitutes green algae in which the cell walls contain a large quantity of mercerized-like cellulose, which is presumably a derivative of native cellulose. This cellulose has a low degree of crystallinity, and the chains are randomly oriented. The bulk of algae fall into this category, including the Spongomorpha. Group 3 is a small group of heterogeneous algae in which cellulose is not the major component of the cell walls or that shows such abnormalities in XRD patterns that any identification becomes difficult. Prominent among these algae are Vaucheria and Spirogyra algae.

The cellulose derived from marine green algae, for example, from *Valonia* or *Cladophora*, is featured with an exceptionally high degree of crystallinity.[27,28] The degree of crystallinity may be as high as 95%, as obtained from XRD. The distinctive properties of highly crystalline cellulose in XRD are the very well-resolved and narrow peaks, especially at 2θ's of 14 and 16°, which are not common for the native cellulose obtained from higher plants (see Fig. 2). Synchrotron-radiated XRD and neutron diffraction studies have also attested to the presence of characteristic 2θ peaks at 14 and 16° in cellulose samples of *Cladophora* and *Valonia* origin[29]; this indicated a specific uniplanar orientation of the algal cell wall.

The unit cell parameters obtained with electron diffraction from *Cladophora* sp. algae cellulose were $a = 0.670$ nm, $b = 0.595$ nm, and $c = 1.036$ nm (chain axis), $\alpha = 118°$, $\beta = 114°$, and $\gamma = 79.7°$ for the one-chain (triclinic Iα) cell (with over 25 calculated independent reflections) and $a = 0.798$ nm, $b = 0.809$ nm, $c = 1.036$ nm, and $\gamma = 96.8°$ for the two-chain (monoclinic Iβ) cell (with over 16 calculated reflections).[30] On the basis of the electron diffraction results, it was further suggested that these two allomorphs coexist in each *Cladophora* microfibril, alternating either longitudinally or laterally. The transition zone between the two phases was found to be the interface between adjacent H-bonded molecular sheets (viz., 0.39-nm lattice planes).
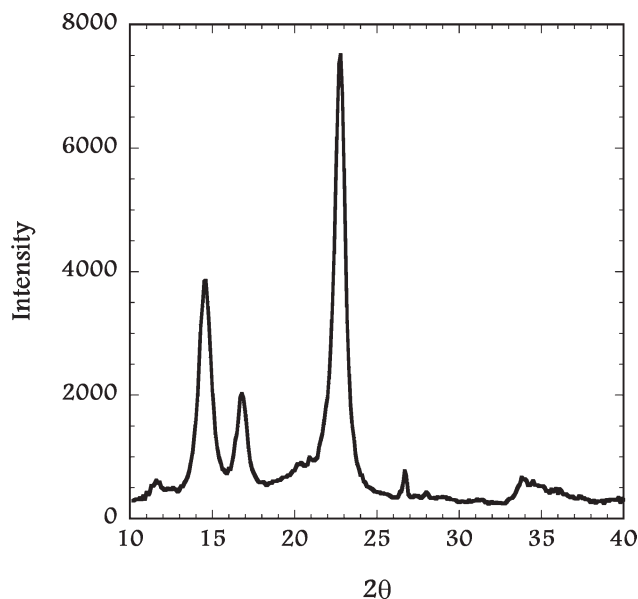

Figure 2 XRD pattern of the *Cladophora* cellulose powder.

The high degree of crystallinity is believed to be related to presence of thick microfibrils.[31–33] The cellulose microfibrils of algal origin are 10–30 nm[28] in width as compared to microfibrils of cellulose derived from wood, which are 5 nm in width.[31,34] The results by Koyama et al.[35] further confirmed that the degree of orientation related closely to the lateral dimension of the cellulose microfibrils. Therefore, marine green algae, such as *Boergesenia, Cladophora,* and *Valonia,* are featured with broad microfibrils with a high degree of orientation, whereas wood cellulose is rich in randomly oriented microfibrils, although both green algae cellulose and wood have a squarelike cross section of microfibrils.[36,37] This fact may indicate that the thin microfibrils can easily twist and form a wavy texture, as observed in the secondary walls of land plants such as wood.[38,39]

The structural differences of the cellulose microfibril dimensions between terrestrial and algal sources could be traced to the cellulose synthase complexes [also called terminal complexes (TCs)], which determine the size of the cellulose microfibrils.[32,40,41] By analyzing the ratio of Iα to Iβ in relation to the cellulose TC's morphology, Vander Hart and Atalla[42,43] reported that linear TCs usually produce Iα-rich cellulose, whereas rosette TCs produce Iβ-dominant cellulose, although a boundary type between Iα-rich and Iβ-dominant celluloses might exist in certain species of green algae (*Chlorophyta*).[44]

According to Koyama et al.,[35] there is an intrinsic variability in the relative distribution of the two allomorphs of cellulose in algae, and three types of cellulose can be identified in general: Iα-dominant broad microfibrils (0.6-nm oriented type), Iβ-dominant flat ribbons (0.53-nm oriented type), and Iβ-

dominant small (random-oriented type). The first type appears to occur in more primitive organisms than the other types, such as marine green algae. The three types of algal cellulose correlate well with the arrangement of cellulose synthase complexes, that is, multiple-row linear types, consolidated rosette types, and isolated rosette types, respectively. For *Cladophora, Boergesenia*, and *Valonia* algae, the 0.6-nm-type cellulose is typical. Thus, in all higher plants, the cellulose synthases appear mainly as consolidated solitary rosettes of six hexagonally arranged subunits, producing thin microfibrils with a random orientation. In contrast, the synthases of certain marine green algae species, such as *Valonia, Cladophora*, and *Boergesenia*, are mainly arranged into linear-type TCs capable of producing very thick microfibrils with a high degree of orientation. For an extensive review, see the article by Tsekos.[45]

The apparent (true) density of *Cladophora* cellulose is around 1.64 g/cm^3, whereas that of cellulose from land plants is on the order of 1.56 g/cm^3, as measured by He pycnometry.[27] The relatively higher apparent density of *Cladophora* cellulose suggests a higher degree of cellulose crystallinity, which is in accordance with results from other analytical techniques. The high degree of crystallinity of algal cellulose results in a higher inertness of cellulose and makes this type of cellulose even less susceptible to various chemical treatments than its more conventional native cellulose analogues derived from land plants. By considering the apparent (true) density of *Cladophora* cellulose and the specific surface area of the material obtained from N$_2$ gas adsorption, Ek et al.[27] estimated the radius of *Cladophora* fibers from geometrical considerations of a cylinder. The obtained value of 38 nm was very close to the dimensions obtained by atomic force microscopy analysis and also to previously reported literature values. Atomic force microscopy in combination with transmission electron microscopy (TEM) was used previously to obtain atomic resolution data of repeating cellobiose units in highly crystalline algal cellulose.[46]

It is generally assumed that in cellulose fibrils, ordered regions alternate with less ordered (para-crystalline) regions.[47,48] A method for quantifying the states of order found within cellulose I, based on cross-polarization/magic angle spinning ^{13}C-NMR in combination with spectral fitting, was reported by Larsson and coworkers.[33,49] The most informative region in an NMR spectrum of cellulose I was found to be a signal cluster with a distribution between 80 and 92 ppm.[50] This region contains fairly sharp signals corresponding to C-4 carbons situated in crystalline cellulose Iα and Iβ domains together with disordered cellulose.[33] More specifically, the C-4 carbons of disordered regions are distributed in a broad band and range from 80 to 86 ppm. On the basis of the NMR spectral analysis of *Cladophora* cellulose, cotton linters, and birch pulp, Wickholm et al.[51] concluded that the disordered regions of cellulose exist at the contact surfaces of cellulose crystallites and, thus, represent that part of the crystallite surface that is accessible to various reagents. The latter is of importance for understanding the water–cellulose interactions in *Cladophora*, as discussed later.

Having a high degree of crystallinity, *Cladophora* cellulose tends to absorb very little moisture from the air at ambient relative humidity.[52] This is because the moisture in cellulose is predominantly absorbed in the disordered regions of the cellulose fibers.[53] Therefore, the surface area of ordinary cellulose material measured from moisture sorption isotherms is orders of magnitude larger than that measured with nitrogen because nitrogen does not interact with cellulose and cannot penetrate its bulk.[54] The surface area values obtained from moisture sorption isotherms and those of nitrogen adsorption are of similar magnitude because the cellulose obtained from *Cladophora* algae is almost entirely crystalline.[52] Multilayer fractal Brunauer–Emmett–Teller analysis of moisture sorption isotherms showed that for highly crystalline *Cladophora* cellulose, water–cellulose interactions take place mainly on fibrils neighboring open pores without causing significant swelling, whereas in less ordered cellulose, water is absorbed in the bulk, which causes the pore volume to expand between one and two orders of magnitude.[55] Furthermore, the capillary condensation fractal analysis of moisture sorption showed that water is distributed in a highly tortuous pore network when it is absorbed in the bulk of cellulose particles, whereas in the case of highly crystalline cellulose, for which no swelling is observed during moisture sorption, the pore network is of low tortuosity.[56]

It is well known that never-dried fibers of cellulose are superior to their once-dried analogues, as the latter cannot be restored to their original state. This is a result of a process commonly referred to as the *hornification* of cellulose.[57] Upon drying, the fibers of cellulose agglomerate to form compact particles of low surface area, which cannot be redispersed to their original state. This feature is common in nearly every kind of native cellulose, whether it comes from higher land plants or bacteria.[58] Unlike other types of cellulose, the extent of hornification of cellulose derived from *Cladophora* algae is remarkably small, and this material is redispersible under high-shear homogenization.

The specific surface area of *Cladophora* cellulose powder, as measured by N$_2$ gas adsorption, may be as high as 95 m^2/g,[52] which is comparable to that of industrial adsorbents, which have specific surface
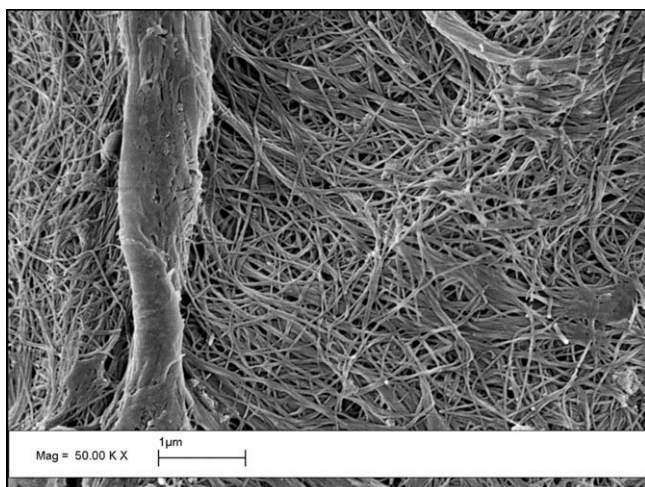
Figure 3 SEM picture of *Cladophora* cellulose at 50,000× magnification.

areas of 100 m^2/g and higher.[59] For comparison, the specific surface area of microcrystalline cellulose (MCC) used widely in pharmaceutics is merely around 1 m^2/g. In scanning electron microscopy (SEM) pictures, a typical weblike structure is seen with numerous intertwined fibers, which are about 20–30 nm in width. Figure 3 shows a typical SEM picture of *Cladophora* cellulose.

In the past, attempts have been made to produce a cellulose material with a large surface area. High-surface-area cellulose aerogel structures can be produced from crosslinked derivatives of cellulose, for example, cellulose acetate or cellulose butyrate, with surface areas as great as 250–389 m^2/g.[60,61] The process of manufacturing of these aerogels includes the dissolution of polymer chains, their crosslinking, and a solvent-exchange, critical-point drying process via the subsequent substitution of water with organic solvents. The process is not only tedious but also difficult for industrial scale-up. However, the biggest disadvantage of this process is that as soon as such aerogels come into contact with a high relative humidity, they absorb large quantities of moisture; this eventually results in a loss of structure due to shrinkage.

One native cellulose material, which is commonly known as *microfibrillated cellulose* (MFC),[62] is interesting in this respect. MFC is a hydrogel of cellulose from the pulp and paper industry and consists of relatively long cellulose fibers. For comprehensive reviews on various types of MFC, see the articles from Siro and Plackett[63] or Eichhorn et al.[64] Nowadays, MFC is often produced by mild enzymatic (monocomponent endoglucanase) hydrolysis followed by high-shear mechanical homogenization.[65] Mild enzymatic hydrolysis is preferred to acidic hydrolysis with mineral acids, which is used to produce other native cellulose varieties, namely,

MCC, because strong acids drastically shorten the fibril length of land-plant cellulose. Freeze-dried aerogels of MFC have recently been reported in the literature, featuring a surface area as high as 70 m^2/g.[66] However, these, like many other processed cellulose types, collapse upon exposure to moisture to form a cellulose powder of low specific surface area upon drying. Unlike hygroscopic cellulose aerogels, no loss of structure or decrease in the high surface area were observed in *Cladophora* cellulose, even after several moisture adsorption–desorption cycles.

MECHANICAL PROPERTIES OF *CLADOPHORA* ALGAE CELLULOSE

Its high degree of polymerization, the linear orientation of its molecules, and its ability to form microfibrils through hydrogen bonds give cellulose exceptionally good mechanical properties. On a molecular level, its strength is related to both covalent bonds and intramolecular hydrogen bonds,[67] which make cellulose an ideal natural component to provide strength to the cell walls in plants. Both *Cladophora* cellulose and cellulose obtained from land plants exhibit a predominantly elastic response to an applied sinusoidal stress.[68] On the molecular level, the strain distribution under load proceeds via the glucose ring, the C—O—C linkage between these rings, and the O(3)→O(5) intramolecular hydrogen bond, whereas the O(2)→O(6) intramolecular hydrogen bond seems to play a minor role. These features are typical both for *Cladophora* cellulose and that obtained from land plants, although distinct variations for the Iα and Iβ allomorphs were observed by dynamic Fourier transform infrared spectra under load.[68]

It is commonly perceived that algae in general do not require strong cell walls as do land plants such as trees. Early investigations revealed that the cell walls in *Cladophora* consist of layers in which cellulose chains in any one layer are inclined to those in the preceding and subsequent layers at an angle of nearly 90°.[69] The natural reason for the presence of highly crystalline cellulose microfibrils in the cell walls of marine green algae featured with exceptionally high density is not clear. It has been suggested that strong microfibrils in *Cladophora* cell walls are necessary to withstand the drag flow from water.[70] Alternatively, strong cellulose microfibrils may be needed to maintain the turgor (i.e., osmotic pressure inside cells) in an environment that features fluctuating salinity.[71]

The tensile properties of algae may depend on the period in the season when the algae are harvested. The cell walls in early season specimens may not be fully developed, and hence, algae collected in this period may exhibit relatively low strength

properties. Similarly, cell walls in algal filaments obtained late in the season may begin to age, and therefore, these algae may also possess low tensile properties. Peak tensile properties can, thus, be expected in algae collected midseason. Johnson et al.[72] showed that *Cladophora* algae extracted early in the season had smaller filaments than a batch obtained midseason; these samples had tensile strengths values of 4 and 9 MPa, respectively. Similarly, aged *Cladophora* algae collected during the late season showed lower tensile strengths than those obtained midseason.[72]

APPLICATIONS

There is a long industrial tradition of harvesting brown and red algae; the estimated value for the variety of products derived from algae amounts to hundreds of millions U.S. dollars.[73] The early industrialization of algae began with the production of soaps or glass from their high contents of soda and potash. Some algae, such as *Laminaria*, were also known for their high iodine content and were, therefore, used as dietary supplements for people suffering from various thyroid dysfunctions.[74] Currently, the three most common industrial derivatives of algae are alginates, agar–agar, and carrageenan used, for example, as hydrocolloids in foods, cosmetics, and pharmaceuticals. There seems, however, to be very little commercial exploitation of green filamentous algae. The cellulose content in filamentous green algae is significant and can be as high as 20–30 wt %.[75,76] Some authors claim that the cellulose content in *Cladophora glomerata* is as high as 45 wt %.[77]

To address the eutrophication problems of coastal areas, it was suggested earlier that green filamentous algae be processed to make fertilizers or soil-improving material in parks, golf courts, and so on. However, the socioeconomic profitability of such enterprises was found to be low.[16] In recent years, there has been a new rapidly expanding technology developed that allows one to obtain biodiesel fuel from algae rich in unsaturated fatty oils.[78] In this respect, some studies have been done on the feasibility of using *Cladophora* and other macroalgae for the production of algae biodiesel.[79,80] The total oleaginous content of *Cladophora glomerata* was reported to be 15.6% per dry weight.[81] For the commercially viable production of algae biodiesel, an oleaginous content of no less than 50 wt % would be necessary, and it seems that microalgae are more suitable for this purpose than macroalgae such as *Cladophora*.[78]

Nonetheless, a range of high-tech applications exists in which the properties of *Cladophora* algae could lead to highly viable economical solutions; this is discussed in more detail later.

Cladophora algae as fish food

Cladophora algae have been shown to be a valuable protein source for feeding herbivorous fish.[82] In general, only 5% of all fish species are herbivorous, and 89% of all herbivorous fish are from tropical waters.[83] It is believed that the ability to digest algae in herbivorous fish comes from special microorganism flora present in their intestines.[84] Algal meals are especially attractive in many developing countries, where other food materials are scarce and the labor required for harvesting and processing is relatively cheaper.[82] Currently, there are small communities in tropical countries of southeast Asia that cultivate *Cladophora* algae as a fish food supplement, for example, for milkfish (*Chanos chanos*).

Cladophora algae cellulose as reinforcement fibers in construction materials

Johnson and Shivkumar[70] reported the use of *Cladophora* algae fibrils as reinforcements in polyurethane foams. It was previously shown that cellulosic materials such as wood flour are inexpensive, readily available, and compatible with polyurethanes.[85] It was suggested that hydroxyl groups on the surface of cellulose fibers exhibit a high chemical affinity to polyurethane and, thus, may act to covalently bond the polyurethane matrix with the cellulosic filler.[86] The reinforcement of polyurethane foams by cellulose fibers serves the purpose of improving the elastic modulus, increasing the impact strength, enhancing the thermal resistance, controlling color, improving the biodegradability, and decreasing cost. Figure 4 shows electron micrographs of polyurethane foams reinforced with *Cladophora* algae fibers. The length of *Cladophora* fibers was found to have a significant effect on the foam density, and peak mechanical properties were obtained for *Cladophora* algae contents of 5–10 wt %.[70] However, in the study by Johnson and Shivkumar,[70] instead of purified cellulose fibers, comminuted algae flour was used. Therefore, provided that the cellulose is purified from algae and properly dispersed, the reinforcement strength of polyurethane foams by *Cladophora* cellulose fibers is expected to be significantly improved.

Cladophora algae cellulose as a filter medium

Cellulose is one of the most commonly used substrate materials in filter membranes, including a wide range of products spanning from ordinary filter paper to dialyzer membranes. Obtaining cellulose fibers 20–30 nm in diameter is challenging. Currently, to obtain cellulose fibers in the this size range, the industry dissolves cellulose mass from the
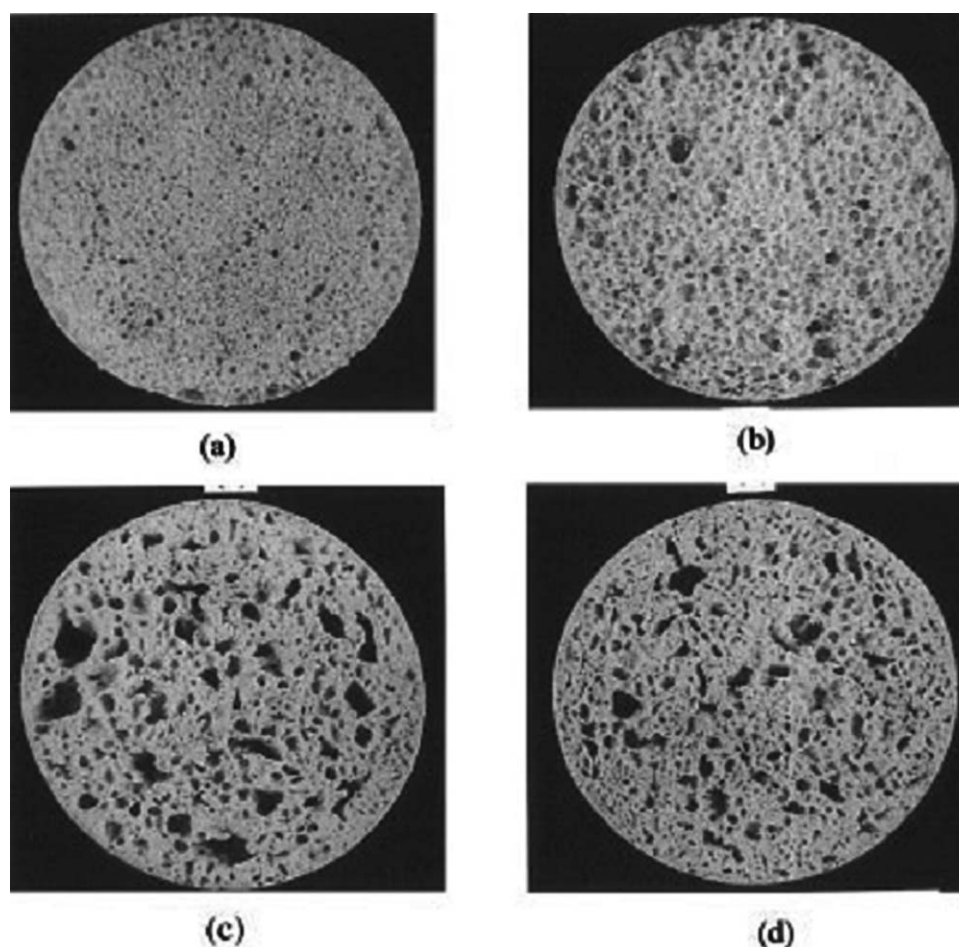
Figure 4 Photographs showing the structure of the polyurethane foams with various amounts of algae: (a) 2.5, (b) 6.5, (c) 13, and (d) 20 wt %. In each case, the algal filaments were dried and ground to obtain an average fiber length of about 600 μm.[70]

pulp and paper industry in suitable solvents first and then regenerates the cellulose.[87] *Cladophora* cellulose is a naturally occurring cellulose material with fiber dimensions highly demanded by the filter materials industry. Two patents for filtering media using *Shiogusa* seaweed, which is the local name for *Cladophora* green algae,[90] were published by Mitsubishi Paper Mills, Ltd.[88,89]

Cladophora algae cellulose as a drug carrier

MCC, which is the purest form of commercially available native cellulose, is one of the most commonly used drug excipients. The large surface area of *Cladophora* cellulose and its inertness make this material an alternative to MCC as a drug carrier. Purified, mineral-acid-hydrolyzed *Cladophora* cellulose forms stronger tablets compared to its commercially available analogues.[91,92]

Cladophora cellulose is also a promising carrier material for liquid drugs. Its large surface area is highly useful for obtaining a high degree of loading of liquid pharmaceuticals, for example, nicotine.[93] When stored inside the pores of *Cladophora* cellulose,

nicotine is protected against oxidative degradation. The results of a 3-month stability study showed that nicotine remained almost intact when loaded inside *Cladophora* cellulose powder particles, unlike when in was loaded inside ordinary MCC blends, in which case a very high level of nicotine degradation products was observed: the values were 0.9% in *Cladophora* cellulose versus 23.6% in MCC blends.[93]

Rheology enhancers based on Cladophora algae cellulose

Water-based dispersions are ubiquitous (foods, cosmetics, drugs, paints, etc) and require viscosity enhancers to prevent the sedimentation of dispersed matter. Sugars and polyols can be used for this purpose, but to achieve the desired effect, large quantities should be used. Alternatively, a number of hydrocolloids in small quantities can be used to attain the desired viscosity; however, these are usually sensitive to temperature and other factors, such as electrolyte content. Nanosized crystals (>60% of particles < 100 nm) of MCC in combination with medium-molecular-weight carboxymethylcellulose (CMC) have proven useful

in the stabilization of various dispersive systems.[94,95] This is because nanocrystals of MCC (the major component) are water-insoluble and, therefore, render the system with desirable thermal stability; this makes this material superior over other water-soluble viscosity enhancers.

Traditionally, MCC/CMC grades are used to obtain rheology enhancers. MCC is attrited to produce crystallites by means of high-shear rubbing in aqueous media. During attrition, new surfaces are formed as the crystallites are separated. If no protective hydrocolloid is used, the crystallites will reagglomerate upon drying and produce an essentially nonporous, low-surface-area product. The produced dispersible MCC/CMC grades form thixotropic gels at solids content of 1.2% and greater.[94–96] One of the main shortcomings of dispersible MCC/CMC grades is their sensitivity to electrolytes and other competitors for water, in general. Furthermore, whereas conventional dispersible MCC/CMC grades have been used to substantially reduce fat in food, large quantities of cellulose-based ingredients must be added to obtain a desirable consistency; this imparts adverse organoleptic properties. Depending on the product, the adverse effects could include a drying sensation, chalkiness, or astringent or other disagreeable flavor.

Investigations have revealed that *Cladophora* cellulose forms stable gel structures at significantly lower solids contents than ordinary dispersible MCC/CMC grades.[97] To achieve a gel strength of similar magnitude, about 10 times less cellulose powder is needed than ordinary MCC/CMC powder. The Cox–Merz complex dynamic viscosity plots for *Cladophora* cellulose dispersions above 0.2 wt % were linear as a function of applied frequency, which is typical for true gel systems, and the values of complex dynamic viscosity were in the range between 10 and 10^6 Pa s for frequencies between 10 and 10^{-3} Hz, respectively.[97]

The threshold value for the formation of gel structures was found to be about 0.2 wt % for *Cladophora* cellulose. Also, less CMC was needed as a dispersion aid for *Cladophora* gels compared to commercial analogues. Furthermore, a *Cladophora*/CMC cellulose dispersion (e.g., 0.5% solids content per volume) does not coagulate in the presence of electrolytes, even when the concentration of electrolyte exceeds 10% w/v.[97] Commercial analogues coagulate when the calcium chloride content is at 4% w/v with characteristic phase separation. Even if the electrolyte does not completely dissolve in *Cladophora* cellulose gels, the salt grains remain suspended in the viscous mass, which does not change its appearance.

To form gels of *Cladophora* cellulose, energy must be provided to activate its individual fibrils. High-
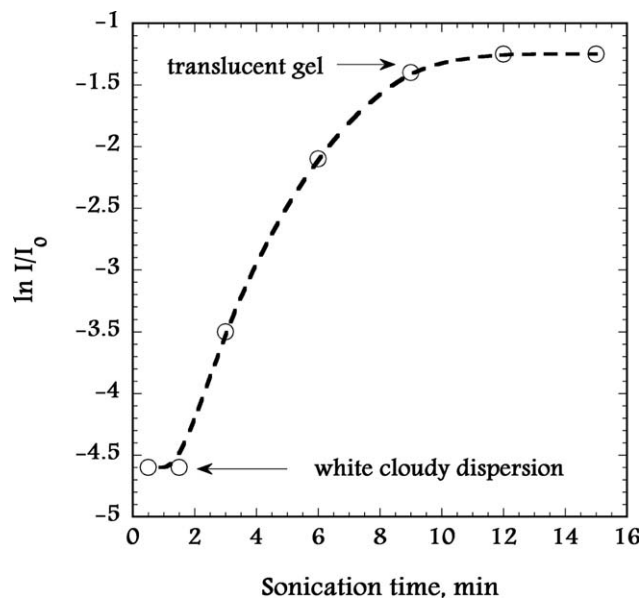

Figure 5 Relative transparency (Rose photometry) of the activated *Cladophora* cellulose dispersion (5.7 $\pm$ 0.3 mg/ 10 mL) as a function of sonication (600 W, 20 kHz) time [I = transmission through water (%); I_0 = transmission through dispersion (%)].

energy sonication can be used to achieve this goal. Figure 5 shows the change in the transparency of *Cladophora* cellulose dispersion as a function of sonication time, which in turn, is proportional to energy input. As shown in this plot, the dispersion becomes progressively transparent until a plateau is reached. The visual transition of the *Cladophora* cellulose dispersion is from a white cloudy mass suspended in water at the start of sonication to a translucent viscous gel after a sufficiently long treatment.

The rheological behavior of *Cladophora* cellulose is somewhat similar to that of MFC and is probably due to the longer fibers in both materials. The solids content threshold for the gel formation of MFC is approximately the same as for *Cladophora* cellulose.[98] However, because MFC is produced by mild enzymatic hydrolysis, it is sensitive to the presence of strong mineral acids, whereas highly crystalline *Cladophora* cellulose is very robust and is not susceptible to chemical reactions. The robustness of *Cladophora* cellulose together with its excellent rheological properties make this material an interesting possibility for applications in foods, pharmaceuticals, paints, wound dressings, and reinforcements in biodegradable plastics.

Conductive Cladophora algae cellulose composites

The high surface area of *Cladophora* cellulose powder makes this material a suitable substrate for the manufacture of highly porous composite paper materials. Cellulose is generally considered to be a material
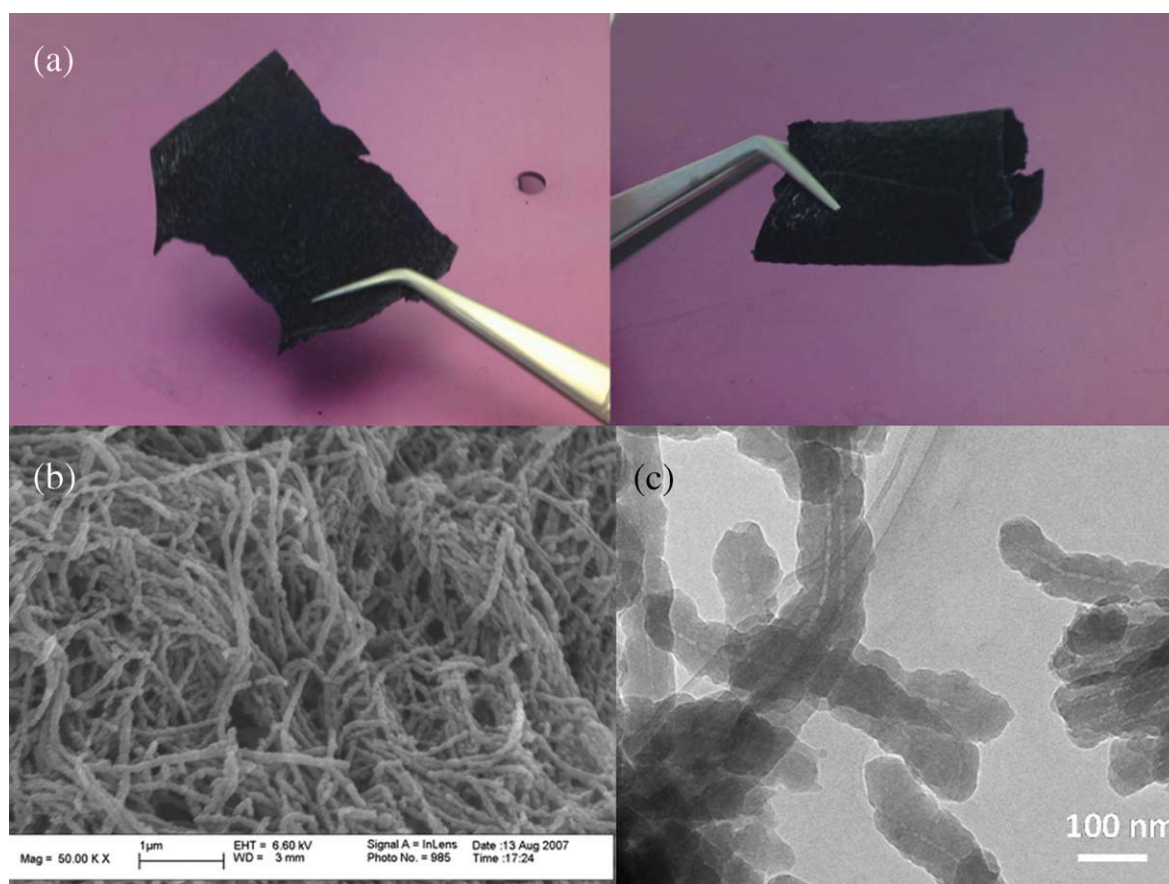
Figure 6 Paper sheet produced by the polymerization of PPy on *Cladophora* cellulose (a). The sheet could be foiled without impairing the integrity of paper. SEM picture of PPy-functionalized *Cladophora* cellulose fibers and TEM image of a typical PPy-coated *Cladophora* cellulose fiber (b). The lighter contrast in the center of the fiber was associated with a single *Cladophora* cellulose fibril, estimated to be about 20 nm in diameter, which was consistent with a PPy coating of about 50 nm in thickness (c). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

with insulating properties. However, when the cellulose fibers are coated with a layer of a conductive polymer, for example, polypyrrole (PPy) or polyaniline, the cellulose can be rendered with conductive properties. These composites can be molded in paper sheets that feature the mechanical properties of paper and the conductive properties of metals.

Composites of cellulose from land plants with conductive polymers have been described.[99,100] The results show that cellulose has a high affinity for PPy and that composites can be readily produced with a direct chemical polymerization process. Many of the applications of PPy are in one way or the other related to ion exchange with the surrounding liquid, for example, in battery applications or electrochemical ion-exchange membranes. This is because different redox forms of PPy are associated with ion inflow or release to maintain the polymer electroneutrality. When PPy is oxidized, the chains become positively charged, and anions enter the film to compensate for charge imbalance. On the other hand, during reduction, the PPy chains become neutral, and anions are released.[101] Thus, when the reduced and oxidized forms of PPy switch, the polymer can function as an ion-exchange membrane.

Recently, a composite paper material consisting of PPy and *Cladophora* cellulose was designed. The composite material had the appearance of black paper sheets and could be bent or foiled without a disruption of its mechanical integrity (see Fig. 6). The material had a specific surface area of 70–80 m^2/g and a conductivity of about 1 S/cm.[102] The thickness of the coating on individual cellulose fibers was uninterrupted and merely 50 nm thick. The thin coating of PPy ensured rapid kinetics of ion exchange, whereas the large surface area ensured a high ion-exchange capacity. The fraction of *Cladophora* cellulose in these composites was 1/3 on the basis of their weight.[103]

The application possibilities for composite conductive *Cladophora* paper are numerous and include gas-sensor devices, ion-exchange membranes, the extraction and purification of biomolecules, diagnostics, and energy-storage devices.

The high surface area of conductive paper composites is especially suitable for so-called batch-wise ion separations, wherein the composite is directly
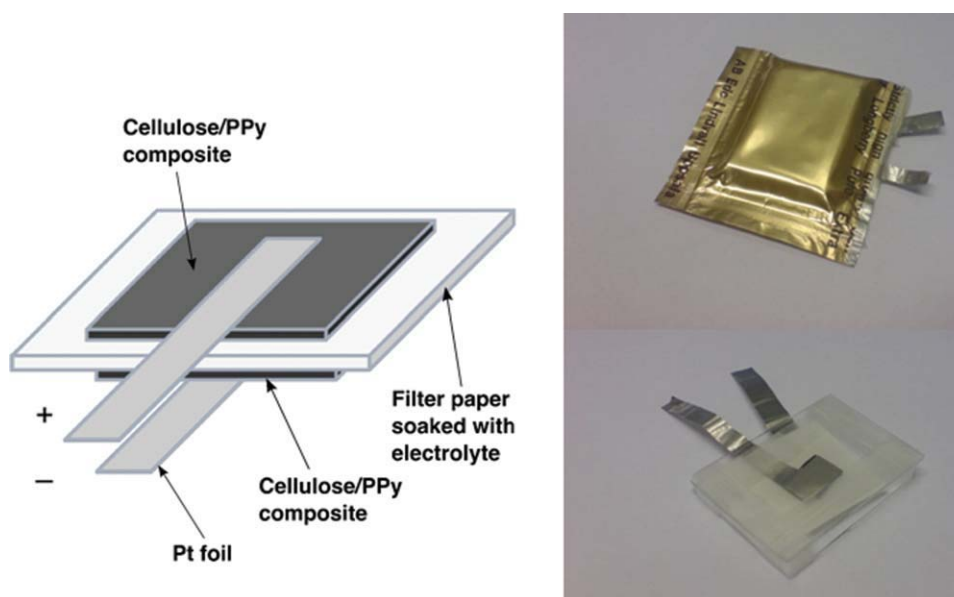
Figure 7 Schematic drawing (left) and photograph (right) of the *Cladophora* cellulose–PPy composite paper battery cell before and after it was sealed in an aluminum pouch.[103] [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

immersed in a solution to be ion-exchanged as opposed to chromatographic columns typically used for ion separation.[104,105] The ions of interest could be both of inorganic nature and biomacromolecules, for example, DNA. The advantage of using the material is that its transition between the reduced and oxidized states via externally applied current is reversible, and thus, the material can be used repeatedly for the extraction and release of ions. Investigations revealed that *Cladophora* composite material exhibited an exceptionally high ion-exchange capacity, both for small inorganic and relatively large organic ions.[104] For instance, the capacity to extract chloride anions was as high as 5 mol of Cl^-/kg of composite.[104]

The material is also of interest for making paper-based energy storage devices. A simple two-electrode battery cell consisting of PPy/*Cladophora* cellulose was recently reported.[103] The electrodes of such a battery consisted of two identical pieces of composite paper, of which one of the electrodes was in the oxidized state and the other one was in the reduced one (see Fig. 7). A piece of filter paper soaked with a 2*M* sodium chloride solution functioned as a separator. The created potential difference between the electrodes due to the state of oxidation was shown to be enough to store energy with an open-circuit voltage of approximately 1 V. The large internal surface area of the composite ensured a large charge capacity in the device, whereas the thin coating ensured rapid charge–discharge. The batteries based on this material were charged with currents as high as 600 mA/cm^2 with only a 6% loss in capacity over 100 subsequent charge–discharge cycles. The batteries exhibited charge capacities

between 25 and 33 mA h g^{-1} or 38 to 50 mA h g^{-1} per weight of the active material. The batteries based on *Cladophora* paper could be charged extremely rapidly, that is, within 11 s at 600 mA/cm^2. The characteristics of the described paper-based battery system are very promising for devices based entirely on nonmetal components. The latter is expected to open up entirely new possibilities for the production of cost-efficient, upscalable, and lightweight energy-storage systems.

CONCLUSIONS AND PERSPECTIVE VIEW

Unlike brown and red algae, which are harvested and processed on a large industrial scale, green algae so far have found only limited industrial use. However, polluting *Cladophora* green algae represent an unexploited, renewable source of highly crystalline cellulose material that features highly beneficial properties that could be used in various industrial applications. Finding high-tech, niche-product applications for *Cladophora* cellulose will clearly raise the awareness about the unique properties of this material and may alleviate serious environmental problems associated with seasonal algal blooms.

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