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Cellulose of some Indian red seaweed species
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I.4.1 INTRODUCTION

Cellulose was discovered by the French chemist Anselme Payen in 1838 and the term cellulose was first time used in a report of the French academy on the basis of his work in 1839 (Klemm et al. 2005). For many years cellulose was used in various forms like cotton, wood and other plant fibers as a source of energy, for building materials as well as for clothing. Cellulose is the most common organic polymer that occurs naturally, consisting of a chain of (1→4)-linked β-D-glucose residues and representing about $1.5 \times 10^{12}$ tons of the total annual biomass production (Klemm et al. 2005). It is considered an important source of raw material for eco-friendly and biocompatible materials as well as for production of ethanol and biofuel (Kreger, 1962; Vander-Hart and Atalla, 1987; Badger, 2002; Teeri et al. 2007; Wi et al. 2009; Kim et al. 2010; John et al. 2011). Cellulosic materials are considered to be an important medium in chiral chromatography (Hesse and Hagel, 1973) apart from their traditional applications. Cellulose exists as a mixture of two crystalline forms, α and β. α-Cellulose, or true cellulose, has a one-chain triclinic structure, whilst β-cellulose has a two-chain monoclinic structure (Sugiyama et al. 1991).

Cellulose has a widespread distribution being found in red, brown and green seaweeds (Naylor and Russell-Wells 1934; Black 1950; Whistler and Charles 1953; Ek et al. 1998; Strømme et al. 2002; Mihranyan et al. 2004). Seaweeds are considered a potential source for cellulose, which is useful in the preparation of various materials (Berglund, 2005). Cellulose from the green alga Chaetomorpha melagonium was found to have some significant variation in X-ray intensity data indicating structural differences over the number of chains within the unit cells (Nieduszynski and Atkins 1970). Crystalline features of algal celluloses were evaluated by Koyama et al. (1997) and were found in 1-20% yields in most of the seaweeds investigated. Recently, we have reported isolation and characterization of celluloses from 33 various Indian seaweed species (Siddhanta et al. 2009, 2011). The high growth rate and plenty of availability of seaweeds may be useful as a potential source of cellulose. The extraction of cellulose from terrestrial plants accompanied with the removal of lignin which greatly influence its processing cost. The removal of lignin from woods has its own importance in the pulp and paper industry posing however a problem by influencing the aquatic ecosystem (Adler, 1977; Roberts, 1996).

In India, more than 800 seaweed species of 29 orders belonging to different classes (Chlorophyta, Phaeophyta and Rhodophyta) have been reported from its ~5700 km long coastal line (Oza and Zaidi 2001; Jha et al. 2009). In this dissertation...
cellulose contents of the red seaweed species belonging to three different taxonomic orders of Indian waters viz. *Champia indica*, *Champia parvula* (Order-Rhodymeniales), *Sarconema filiforme*, *Sarconema scinaioides*, *Hypnea valentiae* (Gigartinales) and *Grateloupia filicina* (Cryptonemiales) were extracted and characterized. This was done as part of a comprehensive study on profiling of cellulose contents of Indian seaweeds in an ongoing initiative in the author’s laboratory (Siddhanta et al. 2009, 2011).

I.4.2 MATERIALS AND METHODS

I.4.2.1 Materials

*Champia indica*, *Champia parvula* (Rhodymeniales, Rhodophyta), *Sarconema filiforme*, *Sarconema scinaioides*, *Hypnea valentiae* (Gigartinales, Rhodophyta) and *Grateloupia filicina* (Cryptonemiales) were collected in April 2007 from Okha (22.28° N, 69.04° E), in April 2007 from Diu (20.42° N, 70.58° E), in March 2008 from Okha, in January 2007 from Veraval (20° 55’ N, 70° 20’ E) in April 2010 from Dwarka from (22.14° N, 68.57° E) and in December 2010 from Madhi (22.03° N, 69.57° E) west coast of India, respectively. Herbaria specimens (AL-II-112-12; AL-II-112-01; AL-II-126-09; AL-II-104-03; and AL-II-159-06) were deposited with CSMCRI Herbaria. Cellulose extracted from Whatman filter paper No. 4 was used as reference (Prasad 2010; Chhatbar 2011). Sodium chlorite, methanol, sodium acetate, sodium hydroxide, hydrochloric acid, sulphuric acid, sodium hypochlorite were used of LR grade and were purchased from Ranbaxy Fine Chemicals Ltd., Mohali, Punjab (India).

I.4.2.2 Isolation of cellulose from seaweeds

Cellulose was extracted from seaweeds as described by Mihranyan et al. (2004) and Siddhanta et al. (2009, 2011). The dried seaweed materials were depigmented and defatted by methanol in a soxhlet apparatus, 100 g of each defatted algal powder was soaked in 1000 ml of acetate buffer containing 36 gm NaClO₂ for bleaching at 60 °C for 3 h. The bleached algal mass was washed with water until the washing showed pH ~ 7. The washed algal mass was treated with 600 ml NaOH (0.5M) solution at 60 °C for overnight. The alkali treated algal mass was washed with water till neutrality, filtered and dried at room temperature. The dried product was re-suspended in 200 ml hydrochloric acid (5% v/v) and was heated up to boiling and resultant slurry was kept overnight at ambient temperature (30 °C), followed by water washing for removing the excess acid, filtered and freeze dried to get cellulose.
Cellulose from the Whatman filter paper No.4 was also isolated and separated following the same method described by Mihranyan et al. (2004). Yields were calculated on the basis of as received dried seaweeds and were mean of three replicates.

I.4.2.3 Fractionation of cellulose

Alpha (α) and beta (β) cellulose fractions from crude cellulose were obtained by employing the method reported in the literature (Whistler 1963; Siddhanta et al. 2009). Dried cellulose (1g) was soaked in 30 ml alkali (17.5% NaOH) solution at 20 °C for 2 h, followed by shaking in every 15 min. The resulting slurry was centrifuged at 8000 rpm for 15 min. The supernatant containing β-cellulose was removed by decanting and α-cellulose was separated after repeated water washing until pH of washings was ca. 7 followed by freeze drying. The β-cellulose was precipitated with 3N H₂SO₄ from the supernatant (till pH~ 7), the mixture was further kept at 80 °C for 10 minute in order to have complete precipitation of β-cellulose. The precipitated β-cellulose was recovered by centrifugation followed by washing with water and freeze drying. Cellulose from the Whatman filter paper No.4 was also fractionated following the same method as described above. Yields were calculated on the basis of as received dried seaweeds and were mean of three replicates.

I.4.2.4 Characterization of cellulose

Based on the higher yield of crude, alpha and beta cellulose, cellulosics of Sarconema filiforme were chosen for FTIR, TGA, CP-MAS ¹³C NMR and SEM analysis. XRD analysis of cellulose samples of the seaweeds were done for determination of crystallinity index.

I.4.2.4.1 FT-IR Spectroscopy

The FTIR spectra of crude cellulose of all the seaweeds samples were recorded on a Perkin-Elmer Spectrum GX FTIR (USA) instrument by taking 5.0 mg of sample in 400 mg of KBr.

I.4.2.4.2 TGA analysis

Thermogravimetric (TGA) analysis of crude, α- and β-cellulose samples of Sarconema filiforme and Whatman filter paper no 4 were was carried out on a Mettler
Toledo TGA system (Switzerland), using a temperature programming from 30°C to 650°C at a heating rate of 10°C/min in N₂ atmosphere.

I.4.2.4.3 CP-MAS ¹³C-NMR

The crude cellulose, α- and β- cellulose of the *Sarconema filiforme* were analysed by solid state NMR (CP-MAS ¹³C NMR) measurements at 25 °C on a Brüker Avance 500 MHz, Spectrometer (Switzerland) at 52.3 MAS, Net spinning was kept 5000 rpm/min. The cellulose obtained from Whatman filter paper was used as the reference sample.

I.4.2.4.4 Scanning electron microscopy

The surface morphology of the cellulose samples was analysed on a scanning electron microscope (SEM) instrument (Carl-Zeiss Leo VP 1430) applying an accelerating voltage of 10 or 20 kV and magnification 1 to 38 K respectively. Each vacuum oven dried samples of cellulose powder were mounted on a sample holder and coated with gold under vacuum prior to the studies.

I.4.2.4.5 X-ray diffraction analysis

Powder X-ray diffractions studies were done with a Philips X’pert MPD X-ray powder diffractometer using 2θ = 5° to 45°. Crystallinity indices (C.I.) were calculated using the following equation (1) (Mihryan et al. 2004; Siddhanta et al. 2009 and 2011; Park et al. 2010).

\[ \text{C. I.} = \frac{I_{002} - I_{am}}{I_{002}} \]  

Where \( I_{002} \) is the overall intensity of the peak at 2θ about 22° and \( I_{am} \) is the intensity of the baseline at 2θ about 18°.

I.4.3 RESULTS AND DISCUSSION

I.4.3.1 Yield of cellulose

The yield of crude cellulose, α-cellulose and β-cellulose, obtained from the various seaweeds as well from Whatman filter paper No.4 are shown in table no I.4.1. It was found that the Whatman filter paper No.4 cellulose was made of mainly alpha
cellulose. The yield of crude, alpha and beta cellulose was higher in Sarconema filiforme (e.g. crude ~ 4.0 %, alpha~ 2.6 % and beta~ 1.1 % w/w) while it was ~2-2.9 % for crude, ~1.0-2.0 % for alpha and ~0.1-0.5 % for beta cellulose in other seaweeds species studied in this dissertation. Alpha cellulose was the main constituents of these seaweeds suggesting that the res algal cellulose can be good source of crystalline cellulose.

Table I.4.1: Cellulose contents of Champia indica, C. parvula, Sarconema filiforme, S. scinaoides, Hypnea valentiae and Grateloupia filicina

<table>
<thead>
<tr>
<th>Seaweed</th>
<th>Crude (%)</th>
<th>Alpha (α) (%)</th>
<th>Beta (β) (%)</th>
<th>α/β ratio</th>
<th>Crude (α) (%)</th>
<th>Alpha (α) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whatman filter paper No. 4</td>
<td>94</td>
<td>88</td>
<td>≤ 1</td>
<td>88</td>
<td>0.79</td>
<td>0.89</td>
</tr>
<tr>
<td><strong>Order- Rhodymeniales</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Champia indica</td>
<td>2.25</td>
<td>1.76</td>
<td>0.38</td>
<td>4.8</td>
<td>0.70</td>
<td>0.72</td>
</tr>
<tr>
<td>Champia parvula</td>
<td>2.10</td>
<td>1.60</td>
<td>0.34</td>
<td>4.7</td>
<td>0.77</td>
<td>0.78</td>
</tr>
<tr>
<td><strong>Gigartinales</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sarconema filiforme</td>
<td>4.01</td>
<td>2.62</td>
<td>1.10</td>
<td>2.4</td>
<td>0.84</td>
<td>0.88</td>
</tr>
<tr>
<td>Sarconema scinaoides</td>
<td>2.10</td>
<td>1.01</td>
<td>0.30</td>
<td>3.3</td>
<td>0.69</td>
<td>0.76</td>
</tr>
<tr>
<td>Hypnea valentiae</td>
<td>2</td>
<td>1.76</td>
<td>0.1</td>
<td>17.6</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td><strong>Cryptomeniales</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grateloupia filicina</td>
<td>2.9</td>
<td>2</td>
<td>0.5</td>
<td>4.0</td>
<td>0.78</td>
<td>0.82</td>
</tr>
</tbody>
</table>

*Yield w.r.to as received dried seaweed and were mean of three replicates; - Not done

I.4.3.2 FT-IR Spectroscopy

The FT-IR spectrum of the crude cellulose of various seaweeds species were recorded and compared that those of the cellulose isolated from Whatman filter paper No. 4. FTIR spectra of all the samples were similar to the cellulose extracted from Whatman filter paper No. 4 (cf. Sun et al., 2005; Siddhanta et al. 2009, 2011). It may be noted that, the IR spectra of the α- and β-cellulose were identical. These bands show that no degradation of cellulose occurred during the isolation and separation process of cellulose. The FTIR spectra of crude, α- and β- celluloses of Sarconema filiforme are shown in Figure I.4.1. The prominent bands were in the range of (KBr, νmax, cm⁻¹): 3435 (O-H stretching), 2928 (C-H str), 1640 (bound H₂O), 1422 (C-H bending) and 1065 (C-O-C bending).

I.4.3.3 TGA analysis

Thermal behaviors of the crude, α- and β- cellulose obtained from Sarconema filiforme as well as from Whatman filter paper no 4 are shown in Figure I.4.2. Crude,
α and β celluloses started to decompose at 200 °C. The cellulose decomposed in two steps, in the first step at temperatures ranging between 200 °C to 320 °C (for crude and beta cellulose) and 200 °C to 360 °C (for alpha cellulose) sharp mass losses ca. 60, 65 and 85% for crude, alpha and beta celluloses respectively (Figure I.4.2b). In the second step, rapid mass loss was observed from 320 °C to 650 °C (for crude and beta cellulose) and 360 °C to 650 °C for alpha cellulose. Cheng et al. (2009) illustrated that the initial weight loss at lower temperature ranging from 200°C to 360°C was due to the removal of small molecular fragments such as hydroxyl and methyl hydroxyl groups. The second weight loss was up to 650 °C showing the degradation of polymeric chains and the six-member cyclic pyranose structure. Since the thermal degradation behavior is affected by some structural features such as molecular weight, crystallinity and orientation, the relatively sharper decrease in weight of α-cellulose at both stages could be due to its higher crystallinity (Um et al. 2004).

I.4.3.4 CP-MAS $^{13}$C- NMR

The CP-MAS $^{13}$C NMR pattern of seaweed cellulose (crude, α and β) and reference cellulose samples were identical. The CP-MAS $^{13}$C NMR of crude, α- and β- cellulose of Sarconema filiforme are shown in the Figure I.4.3. The CP-MAS $^{13}$C NMR of crude, α- cellulose of Whatman filter paper No. 4 are shown in the Figure I.4.4. The chemical shifts are in good agreement with those reported in the literature (Kono et al., 2002; Sun et al., 2005; Witter et al., 2006). The solid state NMR of α- and β-cellulose obtained from Sarconema filiforme showed single broad peak between 70-80 ppm presumably due to the overlapping resonances of C-2, C-3 & C-5 carbons (Kono et al., 2002). In this study it was observed that signal of C-6 was found to be in between 60.20 to 62.88 ppm for α- and β- cellulose and were similar to those reported by Kono et al. (2002; Figure I.4.3). The chemical shift values of the remaining carbons (C-4 & C-1) were also comparable with those reported in the literature (Kono et al., 2002; Sun et al., 2005).

I.4.3.5 Scanning Electron Microscope (SEM)

The surface morphology of the crude, α-, β- cellulose of Sarconema filiforme, crude and α- celluloses of Whatman paper are shown in Figure I.4.5. Presence of clear microfibrous structure for the cellulose samples obtained from the algae Sarconema filiforme, were observed (Figure I.4.5a-c). On the other hand, the cellulose obtained Whatman filter paper showed the presence of blunt structures (Figure I.4.5d-e). Presences of fibrous cellulose in algal samples were accounted on the basis of their
morphology. SEM images of the $\alpha$- and $\beta$- celluloses fractionated from the crude cellulose showed different morphology. The $\alpha$- cellulose as representatively were found to have rough surface structures, while the $\beta$- cellulose showed presence of blunt structure (Figure I.4.5b-c).

I.4.3.6 X-ray diffraction

The crystalline cellulose-I is known to be predominant and native cellulose among seaweeds species and they exhibit the typical diffraction peaks at around 15° and 23° in XRD analysis (Gilbert and Kadla, 1998). Due to lower yield XRD characterization of beta cellulose in all the seaweed samples studied in this dissertation were not done. The crystallinity indexes (CI) of various crude and alpha celluloses of the seaweeds are given in the Table no I.4.1. The XRD diagrams of crude and alpha celluloses of various seaweeds and Whatman filter paper No 4 are shown in Figure I.4.6. The crystallinity index of crude cellulose were lie in between 0.69 ($\text{Sarconema scinaoides}$) 0.84 ($\text{Sarconema filiforme}$) while for alpha cellulose were lie in between 0.72 ($\text{Champia indica}$) to 0.88 ($\text{Sarconema filiforme}$). Higher crystallinity index of these cellulose samples suggest that these can be used for various applications.

I.4.4 CONCLUSION

Cellulose contents of the red seaweed species belonging to three different taxonomic orders of Indian waters viz. $\text{Champia indica}$, $\text{Champia parvula}$ (Order-Rhodymeniales), $\text{Sarconema filiforme}$, $\text{Sarconema scinaoides}$, $\text{Hypnea valentiae}$ (Gigartinales) and $\text{Grateloupia filicina}$ (Cryptonemiales) were extracted and characterized. The yields of crude, $\alpha$ and $\beta$ cellulose were higher in $\text{Sarconema filiforme}$ than those of other species studied herein. This was done as part of a comprehensive study on the profiling of cellulose contents of Indian seaweeds in an ongoing initiative in the author’s laboratory (Siddhanta et al. 2009, 2011). The XRD patterns indicated that $\alpha$-cellulose was more crystalline than the crude cellulose. The results presented in this study may be useful for industrial applications as well as bioprospecting of the seaweeds.
I.4.5 REFERENCES


Figure I.4.1: FTIR spectra of cellulose of (a) Whatman filter paper No 4 (Crude) and (b) Sarconema filiforme (Crude, alpha and beta)
Figure I.4.2: TGA of Crude, alpha and beta cellulosas of (a) Whatman filter paper No.4 and (b) Sarconema filiforme

Figure I.4.3: CP/MAS $^{13}$C NMR spectra of the cellulose of Sarconema filiforme (a) Crude (b) alpha and (c) beta cellulose
Figure I.4.4: CP-MAS $^{13}$C-NMR of crude cellulose of Whatman filter paper No.4

Figure I.4.5: SEM images of celluloses of Sarconema filiforme (a) Crude, (b) alpha, (c) beta and celluloses of Whatman Filter Paper No 4 (d) Crude and (e) alpha
Figure 1.4.6: XRD of Crude and alpha celluloses of (a) Whatman filter paper No.4, (b) *Champia indica*, (c) *Champia parvula*, (d) *Sarconema scinaioides*, (e) *Sarconema filiforme* and (f) *Grateloupia filicina*