Chapter-I.3

Chemical studies on the cellulose of some brown seaweed species
3 Chemical studies on the cellulose of some brown seaweed species

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I.3.1 INTRODUCTION

The most abundant organic substance that occur naturally is cellulose among all other polysaccharide, consisting of a chain of β-(1 → 4)-linked glucose residues (Figure I.3.1) (Staudinger 1932; Klemm et al. 2005). Cellulose is an inexpensive commercial product of biological origin. This is a renewable and biodegradable polymer and is biocompatible. This being a polysaccharide plays important roles in different biological aspects e.g. development, defense in the seaweed life cycle.

Due to the abundance, low cost and easier processability, this has been studied by researchers since long and has been used extensively in various applications. It has been isolated not only from terrestrial plants but also from algae to some extent. In fact algae are considered as a potential source for cellulose, which is useful in preparation of various materials (Berglund, 2005). Cellulose from the green algal genera Valonia, Halicystis, Cladophora, and from brown alga Laminaria have been reported earlier (Whistler and Charles 1953; Ek et al. 1998; Mihranyan et al. 2004). Cellulose polymer obtained from algal species has a porous or sponge like network and this is considerably different from those of the higher plants (Stromme et al. 2002). Cellulose microfibrils and microcrystals have been prepared from banana rachis (Zuluaga et al. 2007). The various crystalline features of algal celluloses were also evaluated by Makiko et al. (1997) and they have reported that the most of the seaweeds contain 1 to 20 % of cellulose. Cellulose exists as a mixture of two crystalline forms, α and β. α- Cellulose or true cellulose has one-chain triclinic structure, while β- cellulose has two-chain monocline structure (Sugiyama et al. 1991). The rheological properties of cellulose hydrogels prepared from cellulose powder of green alga Cladophora sp. were investigated and compared to commercially available cellulose by Mihranyan et al. (2007). This article would provide the guideline for researchers working on seaweed polysaccharides for the cellulose isolation. In this investigation, cellulose was extracted from some brown seaweeds Cystoseira indica, Sargassum tenerrimum, and Padina tetrastromatica following the method described by Mihranyan et al. (2004). Recently, a study on the profiling of cellulose of Indian seaweeds has been done (cf. Siddhanta et al. 2009 and 2010).

I.3.2 MATERIALS AND METHODS

I.3.2.1 Materials
Cystoseira indica seaweed was collected from Diu (20° 42.727’ N, 70° 55.487’ E), the inter-tidal zone of West Coast of India. Sargassum tenerrimum was collected from Veraval (20° 54.875’ N, 70° 20.832’ E), Diu (20° 42.727’ N, 70° 55.487’ E), from the inter-tidal zone in the west coast of India. Padina tetrastromatica from Okha (22° 08.580’ N, 69° 04.254’ E) from the inter-tidal zone in the west coast and Valai island (09° 10.445’ N, 78° 55.55’ E) from the inter-tidal zone in the south east coast of India. Voucher specimens of all these samples have been deposited with the CSMCRI Herbarium. The seaweed was washed with tap water to remove the solid impurities from the plants and were dried in the shade and powdered in a rotating ball mill and stored for the cellulose isolation in plastic containers. Cellulose extracted from Whatman filter paper No. 4 was used as reference. Methanol, sodium chlorite, 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.3.2.2 Isolation of cellulose from seaweeds

Cellulose was isolated from seaweeds following the method described by Mihranyan et al. (2004) and Siddhanta et al. (2009). The dried seaweed materials were depigmented and defatted by methanol in a Soxhlet apparatus, 100g of each defatted algal powder was soaked in 1L acetate buffer containing 36g NaClO2 for bleaching at 60°C for 3h. The bleached algal mass was washed with water until the washing showed pH ~ 7. The washed algal mass was treated with 600ml NaOH (0.5M) solution at 60°C 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 200ml 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 (initial wt. 5g) was also isolated and separated following the same method described by Mihranyan et al. 2004. Yields were calculated on the basis of as received seaweeds.

I.3.2.3 Fractionation of cellulose

Alpha (α) and beta (β) fractions of cellulose were obtained by employing the method reported in the literature (Whistler 1963; Siddhanta et al. 2009). Dried cellulose (1g) was soaked in 30ml alkali (17.5% NaOH) solution at 20°C for 2h, followed by occasional shaking in every 15min. 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 H$_2$SO$_4$ (20 ml) from the supernatant, the mixture was further kept at 80°C for 10 min. 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 depigmented seaweed residue.

I.3.2.4 Characterization of cellulose

I.3.2.4.1 FT-IR Spectroscopy

The FT-IR spectra of all the cellulose samples including cellulose of Whatman filter paper No.4 were recorded on a Perkin-Elmer Spectrum GX FTIR (USA) instrument by taking 10.0 mg of sample in 600 mg of KBr.

I.3.2.4.2 CP-MAS $^{13}$C-NMR

The cellulose, α- and β- cellulose of the Sargassum tenerrimum was analysed by solid state NMR (CP-MAS $^{13}$C NMR) measurements at 20°C on a Bruker 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.3.2.4.3 X-ray diffraction analysis

Powder X-ray diffractions studies were done with a Philips X’pert MPD X-ray powder diffractometer using $2\theta = 10^\circ$ to $45^\circ$. Crystallinity indices (C.I.) were calculated using the following equation (1) (Mihranyan et al. 2004; Siddhanta et al. 2009 and 2010). Cellulose samples were ignited at 800°C ± 10°C for 6 h and percentage of ash contents were calculated based on the weights of oven dried cellulose samples.

\[
C. I. = \frac{I_{002} - I_{am}}{I_{002}}\ \text{----- (1)}
\]

Where $I_{002}$ is the overall intensity of the peak at $2\theta$ about $22^\circ$ and $I_{am}$ is the intensity of the baseline at $2\theta$ about $18^\circ$ showed in equation 1.
**I.3.2.4.4 Scanning electron microscopy (SEM)**

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 20kV and magnification 1 to 38K 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.3.2.4.5 Thermal analysis**

Thermogravimetric (TGA) analysis of α- and β-cellulose samples was carried out on a Mettler Toledo TGA861, Switzerland, all sample tests were conducted in a N₂ purge (40 ml/min) over a temperature range 30–700°C at an increase rate of 10°C/min.

**I.3.3 RESULTS AND DISCUSSION**

**I.3.3.1 Yield of cellulose**

The yields (%) of crude cellulose, α- and β-cellulose samples and the ratio thereof, obtained from different seaweed species are given in Table I.3.1. The yields (%) of crude cellulose in a range of 9.0 to 10.0% and generally the yields of alpha cellulose were greater than those of beta fractions (Table I.3.1). In Whatman filter paper No.4, the yield of crude, α- and β-cellulose were found 94%, 88% and 6% respectively. Yields were calculated on the basis of as received seaweeds.

<table>
<thead>
<tr>
<th>Cellulose</th>
<th>Yield (%) ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>crude cellulose</td>
</tr>
<tr>
<td>Whatman filter paper No. 4</td>
<td>94 ± 1.0</td>
</tr>
<tr>
<td><em>Cystoseira indica</em></td>
<td>9.0 ± 0.4</td>
</tr>
<tr>
<td><em>Sargassum tenerrimum</em></td>
<td>10.0 ± 0.5</td>
</tr>
<tr>
<td><em>Padina tetrastromatica</em></td>
<td>9.5 ± 0.4</td>
</tr>
</tbody>
</table>

*Yield was calculated on the basis of as received seaweeds; Data presented here are mean of triplicate measurement (± SD)*
I.3.3.2 FT-IR Spectroscopy

The FT-IR spectra of cellulose obtained from Cystoseira indica, Sargassum tenerrimum and Padina tetrastromatica are depicted in Figure I.3.2 and compared that with the cellulose obtained from Whatman filter paper No. 4 (Figure I.3.2). FT-IR spectra of both the materials confirmed the identity of the cellulose extracted from seaweeds with that of Whatman filter paper No. 4 (cf. Sun et al., 2005). The IR result of cellulose revealed that there was no degradation of cellulose occurred during the isolation and purification of cellulose. The prominent bands were in the range of (KBr, νmax, cm⁻¹): 3431-3435 (O-H stretching), 2928-2930 (C-H str), 1630-1640 (bound H₂O), 1420-1422 (C-H bending), and 1020-1065 (C-O-C bending) (Figure I.3.2). It may be noted that, the IR spectra of the α- and β-cellulose were identical. These seaweed species produced ashless celluloses.

I.3.3.3 CP-MAS ¹³C- NMR

The CP-MAS ¹³C NMR pattern of seaweed cellulose (crude, α and β) and reference cellulose samples were identical and shown in Figure I.3.3 and Figure I.3.4. The chemical shifts are in good agreement with those reported in the literature (Hiroyuki et al., 2002; Sun et al., 2005; Witter et al., 2006) and presented in Table I.3.2. The solid state NMR of α- and β-cellulose obtained from Sargassum tenerrimum showed single broad peak between 70-80 ppm presumably due to the overlapping resonances of C-2, C-3 & C-5 carbons (Hiroyuki et al., 2002). In this study we have observed chemical shift values between 60.20 to 62.88 ppm for C-6 (Figure I.3.3 and Figure I.3.4), which were similar to those reported by Hiroyuki et al., 2002. The chemical shift values of the remaining carbons (C-4 & C-1) were also comparable with those reported in the literature (Hiroyuki et al., 2002; Sun et al., 2005).

I.3.3.4 X-ray diffraction

The XRD profile of cellulose samples obtained from the seaweeds exhibited typical diffraction peaks at around 22° due to the crystalline structure of cellulose I (crude), which is known to be the native and predominate crystalline structure of cellulose present in algae (Gilbert et al. 1998) (Figures I.3.5 to I.3.8). Furthermore the α- form of cellulose-I is reported to be the dominant polymorph in algal cellulose (Siddhanta et al. 2009). However, the little amount of β-celluloses obtained from the algae were relatively less crystalline than their α-counterpart (Table I.3.3). The XRD profile of crude α- and β-celluloses obtained from brown seaweeds and Whatman filter paper No.4 depicted in Figure I.3.5 and the crystalline index (CI) values in Table I.3.3.
Table I.3.2 CP/MAS $^{13}$C NMR data for cellulose: (a) Whatman filter paper No. 4 cellulose; (b) Seaweed crude cellulose; (c) α-cellulose (d) β-cellulose.

<table>
<thead>
<tr>
<th>Source of cellulose</th>
<th>δ ppm</th>
<th>Assignment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whatman filter paper No. 4 (a)</td>
<td>62.01</td>
<td>C-6 &amp; C-6'</td>
</tr>
<tr>
<td></td>
<td>68.32 &amp; 69.40</td>
<td>C-2, C-2' &amp; C-5, C-5'</td>
</tr>
<tr>
<td></td>
<td>71.19 &amp; 71.84</td>
<td>C-3 &amp; C-3'</td>
</tr>
<tr>
<td></td>
<td>81.08 &amp; 85.81</td>
<td>C-4 &amp; C-4'</td>
</tr>
<tr>
<td></td>
<td>102.69, 101.05 &amp; 107.81</td>
<td>C-1 &amp; C-1'</td>
</tr>
<tr>
<td></td>
<td>109.17</td>
<td>Not assigned</td>
</tr>
<tr>
<td>Crude seaweed cellulose (b)</td>
<td>62.88</td>
<td>C-6</td>
</tr>
<tr>
<td></td>
<td>72.20</td>
<td>C-2, C-3, C-5</td>
</tr>
<tr>
<td></td>
<td>85.94</td>
<td>C-4</td>
</tr>
<tr>
<td></td>
<td>105.2 &amp; 101.69</td>
<td>C-1 &amp; C-1'</td>
</tr>
<tr>
<td>α-cellulose (c)</td>
<td>62.15</td>
<td>C-6</td>
</tr>
<tr>
<td></td>
<td>70.04</td>
<td>C-2 &amp; C-5</td>
</tr>
<tr>
<td></td>
<td>74.32</td>
<td>C-3</td>
</tr>
<tr>
<td></td>
<td>84.64</td>
<td>C-4</td>
</tr>
<tr>
<td></td>
<td>104.7</td>
<td>C-1</td>
</tr>
<tr>
<td>β-cellulose (d)</td>
<td>60.20</td>
<td>C-6</td>
</tr>
<tr>
<td></td>
<td>70.38</td>
<td>C-2 &amp; C-5</td>
</tr>
<tr>
<td></td>
<td>73.89</td>
<td>C-3</td>
</tr>
<tr>
<td></td>
<td>83.23</td>
<td>C-4</td>
</tr>
<tr>
<td></td>
<td>104.5</td>
<td>C-1</td>
</tr>
</tbody>
</table>

Table I.3.3 Crystalline Indices of cellulose

<table>
<thead>
<tr>
<th>Source of cellulose</th>
<th>Crystalline Indices (C.I)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Crude cellulose</td>
</tr>
<tr>
<td>Whatman filter paper No. 4</td>
<td>0.71</td>
</tr>
<tr>
<td>Cystoseira indica</td>
<td>0.67</td>
</tr>
<tr>
<td>Sargassum tenerrimum</td>
<td>0.65</td>
</tr>
<tr>
<td>Padina tetrastromatica</td>
<td>0.67</td>
</tr>
</tbody>
</table>

I.3.3.5 Scanning electron microscope (SEM)

The SEM images of the surface of the crude cellulose isolated from *C. indica*, *S. tenerrimum*, *P. tetrastromatica* and Whatman filter paper were recorded and it was observed that all the celluloses showed different morphologies. The cellulose isolated from *C. indica* appeared to be fibrous in nature (Figure I.3.9a), while those of *S. tenerrimum* and *P. tetrastromatica* wore a clustered look (Fig. I.3.9b-c). On the other
hand, the cellulose isolated from Whatman filter paper looked like having a loosely packed fibrous structure (Fig. I.3.9d) presumably due to the predominance of α-cellulose therein.

I.3.3.6 Thermogravimetric analysis (TGA)

Thermal behaviors of the α- and β- cellulose obtained from crude cellulose of seaweeds and as well as from Whatman filter paper No 4. are presented in Figure I.3.10. 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 360°C sharp mass losses ca. 80 and 60% respectively. In the second step, rapid mass loss was observed from 360°C to 700°C in both fractions of 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 methylhydroxyl groups. The second weight loss was in the range from 360°C to 700°C showing the degradation of polymeric chains and the six-member cyclic pyran structure. Since the thermal degradation behavior is affected by some structural features such as molecular weight, crystallinity, and orientation (Um et al. 2004), the relatively sharper decrease in weight of α-cellulose at both stages could be due to its higher crystallinity, degree of polymerization and compact interwoven structure (Chang et al. 2009). The thermal degradation of α-cellulose from these seaweed species had similar pattern, while small variations of the latter in all β-celluloses were observed presumably due to the differences in their degree of polymerization and crystallinity.

I.3.4 CONCLUSION

This study has unveiled an overall trend on the cellulose profile of a group of Indian brown seaweeds. There were no mentionable variations in the yields of cellulose in these phaeophycean seaweed species. In tune with our previous observation, lowest and highest yields of cellulose were obtained from the carrageenophytic and agarophytyc species respectively (Siddhanta et al. 2009). All celluloses obtained herein were ashless. The XRD and TGA patterns indicated that α-cellulose was more crystalline than the β-cellulose while the latter showed higher thermal stability than the former. The results presented in this study may be useful for industrial applications as well as bioprospecting of the seaweeds.
I.3.5 REFERENCES


Staudinger, H., 1932, in Die hochmolekularen organischen Verbindungen –Kautschuk und Cellulose, (Springer Verlag), (reprinted 1960)


Figure I.3.1 Repeating unit of cellulose (β-(1 → 4)-linked glucose residues)

Figure I.3.2 FT-IR spectra of cellulose of (a) Whatman Filter paper No. 4, (b) C.indica, (c) S. tenerrimum and (d) P. tetrasstromatica
Figure I.3.3 CP-MAS $^{13}$C-NMR of crude cellulose of Whatman filter paper No.4

Figure I.3.4 CP/MAS $^{13}$C NMR spectra of the cellulose samples obtained from *Sargassum tenerrimum* (a) crude cellulose, (b) α-cellulose and (c) β-cellulose
Figure I.3.5 XRD profiles of the cellulose samples obtained from Whatman filter paper No.4 (a) crude cellulose, (b) α-cellulose

Figure I.3.6 XRD profiles of the cellulose samples obtained from Cystoseira indica (a) crude cellulose, (b) α-cellulose and (c) β-cellulose
Figure I.3.7 XRD profiles of the cellulose samples obtained from *Sargassum tenerrimum* (a) crude cellulose, (b) $\alpha$-cellulose and (c) $\beta$-cellulose

Figure I.3.8 XRD profiles of the cellulose samples obtained from *Padina tetrastromatica* (a) crude cellulose, (b) $\alpha$-cellulose and (c) $\beta$-cellulose
Figure I.3.9 SEM images of cellulose of (a) *C.indica*, (b) *S.tenerrimum*, (c) *P. tetrastractoma* and (d) Whatman filter paper No.4
Figure I.3.10 Thermogravimetric analysis of α and β-cellulose fractionated from (a) Whatman filter paper No.4, (b) C.indica, (c) S.tenerrimum and (d) P. tetrastromatica