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Profiling of cellulose content in Indian seaweed species

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ABSTRACT

Cellulose contents were estimated in 12 seaweed samples belonging to different families e.g. red, brown and green, growing in Indian waters. Each cellulose sample was fractionated to yield alpha (α) and beta (β) celluloses. Characterization was done using various analytical tools and results were validated by comparison with those of the cellulose obtained from Whatman filter paper No. 4. The greatest yields of cellulose (crude), α - and β -cellulose were obtained from *Gelidiella acerosa* (13.65%), *Chamaedoris auriculata* (9.0%) and *G. acerosa* (3.10%). *G. acerosa* was also found to contain relatively high amount of α -cellulose (8.19%). The lowest cellulose contents were recorded from *Kappaphycus alvarezii* (2.00%) and *Sarconema scinaioides* (2.1%), while the latter contained the lowest α -, and β -celluloses and α -cellulose contents, while the carrageenophytic and alginophytic algae contain high cellulose and α -cellulose as well as α - and β -celluloses.

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1. Introduction

Cellulose is a naturally occurring polysaccharide and the most abundant organic substance on the earth, which consists of a chain of β -(1 \rightarrow 4)-linked glucose residues (Staudinger, 1932 ; Gilbert and Kadla, 1998; Klemm et al., 2005). Due to the abundance, low cost and easier processability, this has been attracting the attention of the researchers for a long time and it is used extensively in various applications. It can be isolated not only from terrestrial plants but also from algae to some extent (Zuluaga et al., 2007). In fact algae are considered as a potential source for cellulose, which is useful in preparation of various materials (Berglund, 2005). Whistler and Charles (1953) have extracted cellulose for the first time from green algae of the class Valonia, Halicystis, Cladophora, as well as from the brown alga Laminaria followed by many. More recently, Ek et al. (1998) as well as Mihranyan and his co-workers (2004) have also reported extraction of cellulose from seaweeds. Cellulose is crystalline in nature and it exists as a mixture of two crystalline forms, α and β . α -Cellulose has one-chain triclinic structure, while β-cellulose has two-chain monoclinic structure (Sugiyama et al., 1991). Various crystalline features of algal celluloses were thoroughly evaluated by Koyama et al. (1997) and were found in 1-20% yields in most of the seaweeds investigated. The cellulose obtained from algal species contains porous or sponge like network, which is substantially different from the higher plant cellulose (Strømme et al., 2002).

Cellulosic materials are considered to be an important medium in chiral chromatography (Hesse and Hagel, 1973) apart from their traditional applications. Many unique applications require cellulosic materials with higher structural order (Kreger, 1962; Vander-Hart and Atalla, 1987). India has a long coastline extending to 5700 km and is habitat of a numerous species of seaweeds (Meena and Siddhanta, 2006). Apart from being a source of many bioactive molecules, these seaweeds are an important source of various polysaccharides. We report herein the evaluation of seaweeds of Indian waters as a potential source of cellulose in an ongoing program of our laboratory on value addition of seaweeds. In this study 12 seaweed species belonging to different families e.g. Chlorophyceae, Phaeophyceae and Rhodophyceae, commonly designated as green, brown and red seaweeds, respectively, were studied. To the best of our knowledge, this is the first report of profiling of cellulose of Indian seaweed species.

2. Methods

The seaweeds of this investigation were collected from the Indian coasts and the details are: *Chaetomormpha antennina* (Bori de saint-vincent), Kützing and *Chamaedoris auriculata* Børgesen (both from 20.42°N, 70.58°E) belong to the family Chlorophyceae. *Dictyota dichotoma* (Hudson) Lamouroux (08.51°N and 78.14°E), *Dictyota bartayresiana* Lamouroux (9.17°N and 79.15°E) and

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Sargassum tenerrimum J. Agardh (20.54°N and 70.20°E) belong to Phaeophyceae. Gracilaria edulis (S. Gmelin) P. Silva (09.9°N and 78.43°E), Gelidiella acerosa (Forsskål) J. Feldmann and G. Hamel (20.54°N and 70.20°E), Kappaphycus alvarezii (Doty) Doty ex P. Silva com. nov. (09.9°N and 78.43°E), Gracilaria textorii (Suringar) De Toni (22.28°N and 68.04°E), Gracilaria debilis (Førsskål) Børgesen (22.28°N and 68.04°E), Champia indica Børgesen (22.28°N and 68.04°E) and Sarconema scinaioides Børgesen (20.54°N and 17.20°E) belong to the family Rhodophyceae. Voucher specimens of all these species have been deposited with the CSMCRI Herbarium. All the seaweeds were 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 separate 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 and sodium hypochlorite of LR grade were used and were purchased from Ranbaxy Fine Chemicals Ltd., Mohali, Punjab (India).

3. Isolation of cellulose from seaweeds

Cellulose was isolated from the seaweeds following the method described by Mihranyan et al. (2004). Dried algal powder (taking 100 g of each seaweed in separate experiments) was defatted with repeated extraction with MeOH (500 ml×4) in a percolator for a period of eight days at room temperature (two days for each cycle). The defatted algal powder was soaked in 1 L acetate buffer containing 36 g 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 (0.5 M) 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 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. Yields were calculated on the basis of as received seaweeds.

4. Fractionation of cellulose

The α and β fractions from celluloses were obtained by employing the method reported by Whistler (1963). The dried cellulose (1 g) was soaked in 30 ml alkali (17.5% NaOH) solution at 20 °C for 2 h. followed by occasional 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 (residue) was obtained after repeated water washing until pH of the washing was ca. 7, and the product was collected by freeze drying. β-Cellulose was precipitated from the supernatant with 3 N H₂SO₄ (20 ml), the mixture was further kept at 80 °C for 10 min to ensure complete precipitation of β-cellulose. The precipitated β-cellulose was recovered by centrifugation followed by washing with water to make it acid free; finally the product was collected by 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 seaweeds.

5. Characterization of cellulose

The FT-IR spectra of all the cellulose samples were recorded on a Perkin–Elmer Spectrum GX FTIR (USA) instrument. Cellulose, α and β - celluloses of *C. antennina* were characterized by solid state



Fig. 1. Yields (%) with respect to as received seaweed, of cellulose, α - and β -cellulose: (W) Whatman filter paper No. 4; (A) *Chaetomorpha antennina*; (B) *Chamaedoris auriculata*; (C) *Dictyota dichotoma*; (D) *Dictyota bartayresiana*; (E) *Sargassum tenerrimum*; (F) *Gracilaria debilis*; (G) *Gracilaria textorii*; (H) *Gracilaria edulis*; (I) *Gelidiella acerosa*; (J) *Champia indica*; (K) *Kappaphycus alvarezii* and (L) *Sarconema scinaioides*; Insets: (a) α/β cellulose ratios and (b) The crystallinity indices.

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