

Seaweed-based cellulose: applications, and future perspectives

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Abstract

Cellulose is a naturally occurring organic polymer extracted mainly from lignocellulosic biomass of terrestrial origin. However, the increasing production of seaweeds for growing global market demands has developed the opportunity to use it as an additional cellulose source. This review aims to prepare comprehensive information to understand seaweed cellulose and its possible applications better. This is the first review that summarizes and discusses the cellulose from all three types (green, red, and brown) of seaweeds in various aspects such as contents, extraction strategies, and cellulose-based products. The seaweed cellulose applications and future perspectives are also discussed. Several seaweed species were found to have significant cellulose content (9-34% dry weight). The review highlights that the properties of seaweed cellulose-based products were comparable to products prepared from plant-based cellulose. Overall, this work demonstrates that cellulose could be economically extracted from phycolloids industrial waste and selected cellulose-rich seaweed species for various commercial applications.

Keywords: Bioethanol, Cellulose, Seaweed, Microcrystalline cellulose, Nanocellulose

1. Introduction

Cellulose is the most common biomaterial derived from renewable resources such as terrestrial plants, algae, and fungi (Gupta et al., 2019). Structurally, cellulose is a homogeneous polymer made up of β - (1, 4) linked β -D-glucopyranose units (Moon et al., 2011). Cellulose has been attracting researchers' attention for a long time and is used extensively in various applications due to its abundant availability, low cost, and easier processability (Motaung & Liganiso, 2018). Cellulose versatile characteristics such as low weight, non-toxicity, mechanical strength, hydrophilic and hygroscopic nature, biodegradability, and recyclability make it suitable for various commercial applications (Hafid et al., 2021; Zhang et al., 2021; Du et al., 2018). Cellulose and its derivatives such as cellulose ester, cellulose ethers, cellulose fiber, microcrystalline cellulose, and nanocellulose are widely used in paper, textiles, pharmaceuticals, veterinary, cosmetic, food industries, and water treatment (Kassab et al., 2020; He et al., 2020; Arca et al., 2018; Lakshmi et al., 2017). The annual production of cellulose is estimated at 180 billion tons (Sundarraj & Ranganathan, 2018) and, the market demand for cellulose and its derivatives is steadily increasing (Hafid et al., 2021; Zhang et al., 2021). The cellulose industries highly depend on lignocellulosic biomass like wood, cotton, flax, hemp, and Jute for cellulose production (Lavanya et al., 2011). The removal of lignin is the major challenge in extracting cellulose from lignocellulosic biomass (Lee et al., 2014). The harsh chemical treatment to biomass leads to partial degradation of the cellulose (Ververis et al., 2004) and generates effluents with severe environmental concerns. Therefore, easily available and fast-growing biomass, such as seaweeds, are also gaining global attention as an additional source of cellulose.

Seaweeds (Macroalgae) are broadly classified into Chlorophyta, Rhodophyta, and Phaeophyta, based on their pigmentation (Samiee et al., 2019; Kraan, 2013). Chlorophyta consists of green seaweeds that contain chlorophyll a and b as a primary pigment. The Rhodophyta is a group of red seaweeds that contains phycobiliprotein dominantly. The third is Phaeophyta (brown seaweed) contains fucoxanthin dominantly. Seaweeds, being aquatic organisms, contain copious amounts of water in their bodies up to 85% (Badmus et al., 2019), while the rest is represented mainly by organic matter and minerals. The dry matter of seaweeds comprises total lipids 0.5-3.5% (Kumari et al., 2010), proteins 3-50% (Harnedy and FitzGerald, 2011), total carbohydrates 21-61%, and minerals 12-46% (Baghel et al., 2014; Kumar et al., 2011). A higher growth rate, fewer inputs in seaweed cultivation (require no land and fertilizers, pesticides, and freshwater) makes them an attractive option over the terrestrial biomass (Baghel et al., 2017). Owing to this reason, the world production of seaweeds increased by three times in 2018 (32.4 million tons) from 10.6 million tonnes in 2000 (FAO, 2020). Of total seaweed harvest, 97.1% volume came from farmed seaweeds and 2.9% from wild harvest. Certain seaweeds have been utilized in many countries like Costa Rica, Japan, China, and Egypt from prehistoric times as a human diet (Dillehay et al., 2008). The red

seaweed *Porphyra* (Nori) is used to prepare sushi in various countries like Japan, South Korea, United States, and the United Kingdom due to its richness in proteins and vitamins (Merrill, 1993; Chapman, 2013). The phycocolloids (Carbohydrates) such as agar, carrageenan, and alginate are the main commercial products traditionally extracted from seaweed biomass (Khalil et al., 2018). The hydrocolloid yielding seaweeds are divided into three groups. The agar-bearing seaweeds are known as agarophytes, carrageenan-bearing seaweeds are known as carrageenophytes, while alginate-bearing seaweeds are called alginophytes (McHugh 1991). These hydrocolloids are extensively used as an ingredient in various industries ranging from food, beverages, dairy, animal feed, cosmetics, personal care, pharma, healthcare, textile, printing, and paper coating (Baghel et al., 2017). Also, the genus *Ulva* and *Porphyra* members contain significant amounts of storage sulfated polysaccharides such as ulvan and porphyran. Such polysaccharides exhibit various biological activities with the potentials to develop drugs and nutraceuticals (Pereira, 2020). The seaweed-based minerals are yet other components commercially used as fertilizer (Baghel et al., 2020). Apart from these, seaweeds have very little or no lignin helping to recover pure and intact cellulose with greater suitability for biomedical applications (Halib et al., 2017).

The research on seaweed cellulose has begun long ago. Cellulose from green seaweed was first described in 1885 (Mihrianyan, 2011). Cellulose is present in the seaweed cell wall with other diverse polysaccharides such as xylan, mannan, galactan, alginic acid, agar, carrageenan, rhamnose-glucuronate/iduronic; depending on the type of the seaweed groups (Wahlström et al., 2020a; Ciancia et al., 2020; Michel et al., 2010). Chlorophyceae members are known to have a wide array of walls ranging from cellulose–pectin complexes to ones made of hydroxyproline-rich glycoproteins (Domozych et al., 2012). Figure 1 describing the cell walls of green, red, and brown seaweeds where cellulose is present in the cell wall as the fibrillar skeleton material. In green seaweed, such as *Ulva* species- the cell wall is composed of four polysaccharides families. Amid them, water-soluble ulvan and insoluble cellulose as major constituents, and certain hemicellulose constituents such as β -1,4-D-glucuronan and β -1,4-D-xyloglucan present in minor fractions (Fig. 1A) (Lahaye and Robic, 2007). Further, the most abundant fibrillar constituents of the cell walls of Ulvophyceae may change from cellulose to β -mannans to β -xylans during different life cycle phases (Domozych et al., 2012). Red seaweed cell walls made up of cellulose microfibrils, glucomannan, and hemicellulose material, consisted of sulfated glucan and xylogalactans (Fig. 1B) (Lechat, 1998). For instance, β -D-(1 \rightarrow 4), β -D-(1 \rightarrow 3) mixed linkage xylans are known from Nemaliales and Palmariales (Viana et al., 2011). Sulfated galactans are another major sugars present in the cell walls of red seaweeds, present in the form of carrageenan and agar (Usov, 1998). These xylans and galactans are predicted to be interconnected in cell walls (Fig. 1B); however, proper structures are yet to be elusive. Contrary, the fucose-containing sulfated polysaccharides (such as fucoidans) are the major

crosslinking glycan present in cell walls of brown seaweed (Fig. 1C). These sulfated polysaccharides play a role in interlocking the cellulose microfibrils in layers parallel to the cell surface that create the framework of the cells (Deniaud-Bouët et al., 2017; Michel et al., 2010). Another peculiar feature of brown seaweed is alginate, which can be present either in the homopolymer form of d-mannuronic acid /l-guluronic acid or present in an alternative fashion (Fig. 1C). Notably, lignin is either absent in seaweeds (Ge et al., 2011) or present in very low concentrations (Wi et al., 2009) compared to land plants.

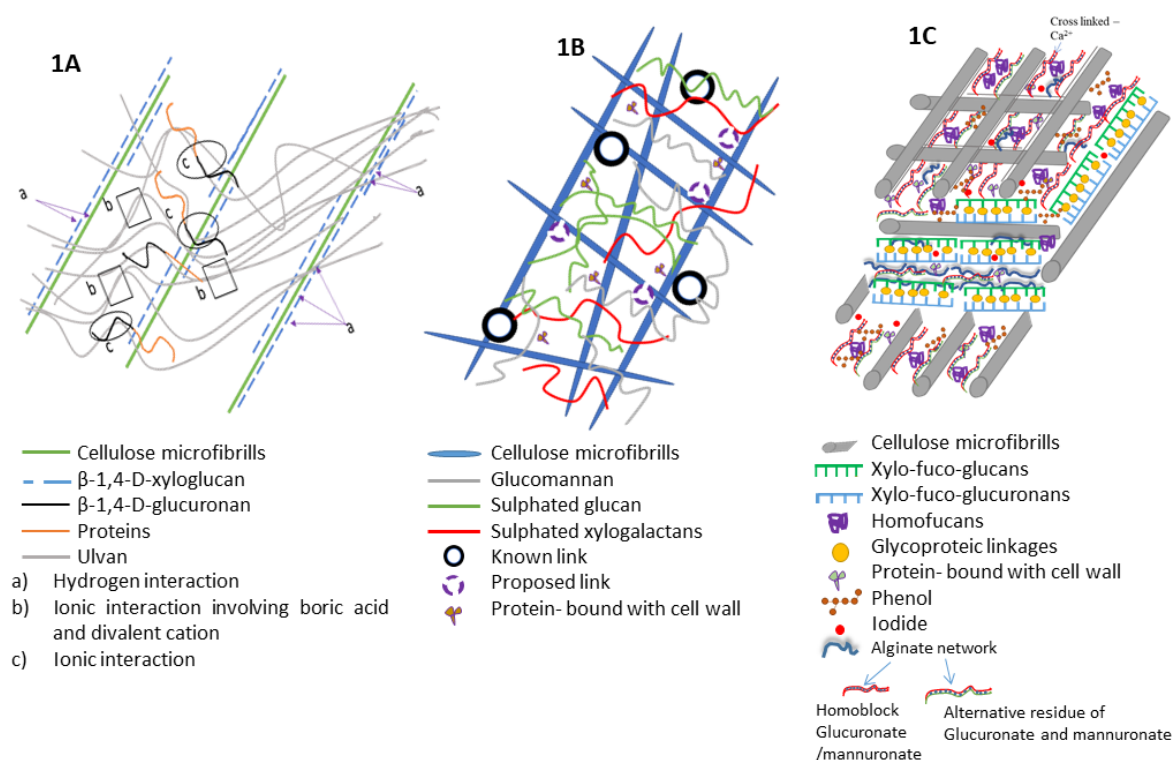


Figure 1 Cell wall composition of seaweeds; 1A, representative cell wall for green seaweeds; 1B, representative cell wall for red seaweeds and 1C, cell wall composition of brown seaweeds. (adapted and modified from Stiger-Pouvreau et al., 2016; Michel et al., 2010; Lahaye and Robic, 2007; Lechat, 1998)

The cellulose sourced from different seaweeds species reported to have similarities with cotton cellulose based on three different analysis, i.e., 1) production of D-glucose only upon hydrolysis, 2) presence of β - (1, 4) linkage confirmed by the preparation of cellobiose octa-acetate and 3) similar X-Ray diagram from algal cellulose and cotton cellulose (Percival & Ross, 1949). However, algal cellulose has differed from cotton/plant cellulose in the compositional ratio of two allomorphs. The algal cellulose is dominated by α allomorph (α cellulose) (Siddhanta et al., 2009) while cotton type cellulose dominated by

β allomorph (β cellulose) (Tsekos, 2002; Horii et al., 1987). Especially the cellulose content and allomorph were analysed in 12 seaweed species belonging to green, red, and brown seaweeds (Fig. 2) (Siddhanta et al., 2009). They found that α allomorph was predominant in all studied species. Interestingly, it showed that species belongs to the agarophytic and alginophytic group contained high cellulose and α -cellulose contents, while the member of carrageenophyte having low cellulose. The dominance of α allomorph in seaweed cellulose makes them more thermodynamically reactive than woody or plant biomass-based cellulose (Siddhanta et al., 2009). Apart from that, seaweed cellulose and plant cellulose reported having a different surface area. Plant cellulose has a specific surface area of 1 square meter per gram; on the contrary, seaweed, *Cladophora* having up to 100 times larger surface area as algal cellulose extruded as single strands (Marsin et al., 2005).

Green seaweeds classified into three groups based on their cell wall constituents (Nicolai & Preston, 1952). Groups 1 comprised the green algae belonging to the two orders, Cladophorales (*Cladophora*, *Chaetomorpha*, *Rhizoclonium*, and *Microdyction*) and Siphonocladales (*Valonia*, *Dictyosphaeria*, *Siphonocladus*, and *Boergesenia*), having the high crystalline native cellulose as the major component of cell walls. The bulk of green algae with a large quantity of mercerized like low crystalline randomly oriented cellulose in their cell walls are classified as Group 2. The small group of heterogeneous algae, in which case cellulose is a minor component in their cell walls like *Vaucheria* and *Spirogyra* included in Group 3. The crystalline features were analyzed for algal celluloses extracted from different species (Koyama et al., 1997), which reported three types of cellulose in the algal system: 1) I_{α} -rich, broad microfibril, 0.6 nm oriented type; 2) I_{β} -dominant, flat-ribbon, 0.53 nm oriented type; and 3) I_{β} -dominant, small, random oriented type. The agarophytic seaweeds were reported to have higher cellulose content than carrageenophytes (Siddhanta et al., 2009). Further, Siddhanta et al. (2011) studied cellulose content and its allomorph for 21 seaweed species belonging to the different classes from Indian waters. They established a relationship between the morphology of algal thallus and cellulose yields. The algal species with compact morphology have higher cellulose content than succulent morphology.

The properties of seaweed-based cellulose vary with the source species. The cellulose fiber extracted from various seaweed species reported having different lengths and widths. For example, fiber from *Gelidium* (red seaweed) was reported with 0.5-1.0 mm length and 3-7 μm width based on scanning electron microscopy images (Seo et al., 2010a, b). Green seaweed *Cladophora* cellulose was reported with 0.7-2.3 mm length and 1.3-1.6 μm width (Leopold & Marton, 1974). The cellulose fiber extracted from another green seaweed *Rhizoclonium* was having 0.2-3.3 mm fiber length (Chao et al., 2000). Similarly, there has also been a difference in the crystallinity index of the cellulose from seaweeds. For example, *Cladophora* cellulose had 84% (Hallac & Ragauskas, 2011); 67.0% for Kelp cellulose (Liu et

al., 2017a), and 48 % for *Ulva lactuca* (Wahlström et al., 2020b). Further, *Cladophora* based cellulose was reported to have a high degree of polymerization (DP) with a weighted average DP of about 2,000 (Hallac & Ragauskas, 2011). The subsequent studies have also investigated various seaweeds to quantify the cellulose yield (Chen et al., 2016; Baghel et al., 2014; Siddhanta et al., 2013, 2011, 2009; Mihranyan, 2011) (Table 1,2,3).

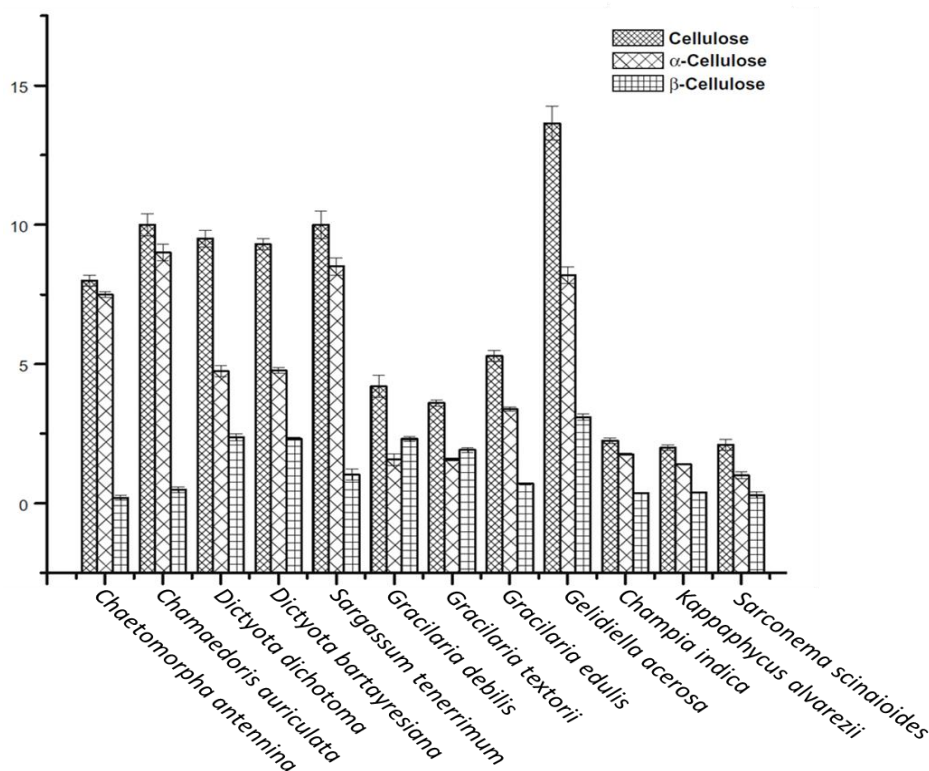


Figure 2 Yields (%) of cellulose, α - and β -cellulose for different seaweed species. (adapted from Siddhanta et al., 2009)

Due to the high ever-growing demands of cellulose and its derivatives, it is essential to identify the additional sources of cellulose along with a sustainable and efficient extraction process (Hafid et al., 2021). In the past decades, seaweeds have been mainly processed to extract hydrocolloids as the major known constituents of biomass (Baghel et al., 2014). However, the last decade has witnessed significant advancements in developing innovative biomass processing technologies facilitating the successful extraction of cellulose from residual biomass leftover following the extraction of minerals, protein, and hydrocolloid (Baghel et al., 2015, Trivedi et al., 2016). The preparation of cellulose from such residual biomass has provided an opportunity for the large-scale extraction of cellulose and its utilization for various applications. With this backdrop, the review is begun with the general introduction of seaweeds and their uses. The presence of cellulose in seaweed cell walls and its similarities and differences with plant-based cellulose are described. Further, cellulose contents in various seaweed species are

summarized. The cellulose extraction strategies are discussed with examples and compared with the cellulose extraction process from lignocellulosic biomass. The standing stock of cellulose in annual phycocolloids industries waste is estimated. Further, seaweed cellulose-based products and their application are reviewed and also compared with products prepared from plant-based cellulose. At last concluding remarks and an outline for future research, programs are provided.

2. Cellulose contents in seaweed

Several species belonging to the green, red, and brown seaweeds have been investigated for their cellulosic content. A total number of 19 species of green seaweeds were studied for cellulose content that ranged from 1.5-34% on dry weight (DW) of biomass with an average being 9.67% (Table 1). Whereas the same in 47 species of red seaweeds ranged from 0.85-18% DW with an average being 4.75% (Table 2). The corresponding value for the brown seaweeds ranged from 2.2-10.2% on a DW basis, with an average being 7.88% based on the investigation of a total of 15 species (Table 3). Overall the cellulose content reported for seaweed biomass ranges between 0.85 to 34%. The investigation on cellulose content from different seaweeds showed that cellulose content not only differs with a different group of seaweed but also among the members of the same group. The member of red seaweed showed higher diversity in cellulose content in comparison to green and brown seaweeds. Among the seaweeds investigated, about 27 species contained cellulose in the range of 9-34 % on a DW basis, indicating a potential opportunity for their commercial cultivation for cellulose production.

Table 1 Cellulose content in green seaweeds on dry weight (DW) basis

S. N.	Order	Species	Cellulose (%)	References
1	Ulvales	<i>Ulva fasciata</i>	11-14.7	Trivedi et al., 2016; Bhutiya et al., 2018., Lakshmi et al., 2017
2		<i>Ulva lactuca</i>	5.6-16.3	Siddhanta et al., 2011; Doh et al., 2020a
3		<i>Ulva rigida</i>	7.5	Siddhanta et al., 2011
4		<i>Ulva prolifera</i>	15.3	Gao et al., 2015
5		<i>Ulva compressa</i>	3.5	
6		<i>Ulva prolifera</i>	5	Siddhanta et al., 2011
7	Cladophorales	<i>Valoniopsis pachynema</i>	6.8	
8		<i>Chamaedoris auriculata</i>	9	Siddhanta et al., 2009

9		<i>Chaetomorpha antennina</i>	8-34	Siddhanta et al., 2011; Bhutiya et al., 2018
10		<i>Chaetomorpha aerea</i>	20	Siddhanta et al., 2011
11		<i>Cladophora glomerata</i>	21.6	Xiang et al., 2016
12		<i>Boodlea composite</i>	5.2	
13	Bryopsidales	<i>Codium dwarkense</i>	1.5	
14		<i>Codium tomentosum</i>	7	
15		<i>Caulerpa taxifolia</i>	11	
16		<i>Caulerpa imbricate</i>	7.4	Siddhanta et al., 2011
17		<i>Bryopsis hypnoides</i>	4.3	
18		<i>Halimeda macroloba</i>	3.7	
19	Ulotrichales	<i>Acrosiphonia orientalis</i>	10.4	
Average cellulose content			9.67	

Table 2 Cellulose content in red seaweeds on dry weight (DW) basis

S. N.	Order	Species	Cellulose (%)	References
1	Nemaliales	<i>Scinaia carnosa</i>	1.2	Siddhanta et al., 2011
2		<i>Liagora ceranoides</i>	0.85	
3		<i>Liagora indica</i>	0.9	Siddhanta et al., 2013
4		<i>Galaxaura oblongata</i>	1.8	
5	Halymeniales	<i>Grateloupia indica</i>	4.2	Siddhanta et al., 2011
6		<i>Grateloupia lithophila</i>	2.9	Siddhanta et al., 2013
7		<i>Grateloupia filicina</i>	2.9	
8		<i>Halymenia venusta</i>	3.6	
9	Corallinales	<i>Cheilosporum spectabile</i>	2.5	
10	Gigartinales	<i>Solieria robusta</i>	2.1	
11		<i>Hypnea valentiae</i>	2	
12		<i>Hypnea pannosa</i>	2	Siddhanta et al., 2011
13		<i>Kappaphycus alvarezii</i>	2	
14		<i>Sarconema scinaoides</i>	2.1	
15		<i>Sarconema filiforme</i>	4	

16	Rhodymeniales	<i>Champia parvula</i>	2.1	
17		<i>Champia indica</i>	2.25	
18		<i>Champia globulifera</i>	2.5	Siddhanta et al., 2013
19	Ceramiales	<i>Laurencia majuscula</i>	3.4	
20		<i>Ceramium cruciatum</i>	2.4	
21		<i>Dasya naccarioides</i>	2.6	
22		<i>Neurymenia fraxinifolia</i>	3	
23		<i>Nitophyllum marginale</i>	1.4	
24		<i>Acanthophora dendroides</i>	3.3	
25		<i>Haloplegma duperreyi</i>	3.4	Siddhanta et al., 2011
26		<i>Laurencia nana</i>	3	
27	Gracilariales	<i>Gracilaria millardetii</i>	3.8	
28		<i>Gracilaria pudumadamensis</i>	2	Siddhanta et al., 2013
29		<i>Gracilaria dura</i>	2.4-3.7	Baghel et al., 2014; Siddhanta et al., 2011
30		<i>Gracilaria edulis</i>	4.88-5.3	
31		<i>Gracilaria debilis</i>	4.2	
32		<i>Gracilaria textorii</i>	3.6-4.62	
33		<i>Gracilaria fergusonii</i>	4.2-4.3	
34		<i>Gracilaria salicornia</i>	4.9	Baghel et al., 2014
35		<i>Gracilaria corticata</i>	5.27	
36		<i>Gracilaria. c. v. cylindrica</i>	6.08	
37		<i>Gracilaria foliifera</i>	3.77	
38		<i>Gracilaria crassa</i>	7.21	
39		<i>Gracilaria verrucosa</i>	14-18	Kumar et al., 2013
40	Gelidiales	<i>Gelidium micropterum</i>	10.63	Baghel et al., 2014
41		<i>Gelidium variabilis</i>	11.38	
42		<i>Gelidium amansii</i>	9.09	Gao et al., 2015
43		<i>Gelidium elegans</i>	17.2	Chen et al., 2016
44		<i>Gelidium pusillum</i>	9.3-12.20	Siddhanta et al., 2011; Baghel et al., 2014
45		<i>Gelidiella acerosa</i>	9.77-13.6	Baghel et al., 2014; Siddhanta et al., 2011

46		<i>Pterocladia heteroplotos</i>	8.7	Siddhanta et al., 2013
47	Bangiales	<i>Porphyra umbilicalis</i>	9.84	Gao et al., 2015
Average cellulose content			4.75	

Table 3 Cellulose content in brown seaweeds on dry weight (DW) basis

S. No.	Order	Species	Cellulose (%)	References
1	Dictyotales	<i>Dictyota dichotoma</i>	9.5	Siddhanta et al., 2011
2		<i>Dictyota bartayresianac</i>	9.3	
3		<i>Padina tetrastromatica</i>	9.5	
4		<i>Dictyota divaricata</i>	5.2	Siddhanta et al., 2013
5		<i>Padina pavonica</i>	6.1	
6		<i>Spatoglossum asperum</i>	2.2	
7		<i>Stoechospermum marginatum</i>	4.5	
8		<i>Pocockiella variegata</i>	9.5	
9	Fucales	<i>Hormophysa triquerta</i>	8.2	
10		<i>Sargassum tenerrimum</i>	10	Siddhanta et al., 2011
11		<i>Cystoseira indica</i>	9	
12		<i>Iyengaria stellata</i>	9.2	
13	Laminariales	<i>Macrocystis pyrifera</i>	5.9	Gao et al., 2015
14		<i>Saccharina japonica</i>	9.9	He et al., 2018
15	Scytosiphonales	<i>Hydroclathrus clathratus</i>	10.2	Siddhanta et al., 2013
Average content		7.88		

3. Cellulose extraction: Primary biomass vs residual biomass

Primarily cellulose is extracted from whole seaweed dry biomass (Mihiranyan et al., 2004; Siddhanta et al., 2013, 2011, 2009; Baghel et al., 2014). The cellulose extraction method presented in Figure 3A includes (1) bleaching of dried seaweed biomass with sodium chlorite (NaClO_2) in an acetate buffer at 60 °C for 3-6 hrs followed by washing to make material neutral. (2) Alkali treatment (0.5 M NaOH) at 60 °C for 6-12 hrs and subsequent washing of material till neutral pH. (3) Heating of alkali-treated material till boiling in 5% HCl (V/V) followed by incubation at room temperature. (4) Washing of material till neutral pH and drying to get cellulose. However, in recent times, integrated approaches have been made to extract cellulose from seaweed biomass along with several co-products (Baghel et al., 2020; Trivedi et

al., 2016; Kumar et al., 2013). For example, 27-35% of cellulose was extracted from residual pulp that leftover after sequential recovery of various components such as pigments, minerals, lipids, and agar from three different agarophytic seaweeds *Gelidiella acerosa*, *Gracilaria dura*, and *Gelidium pusillum* (Baghel et al., 2015). The total cellulose quantity extracted from the respective samples' residual pulp was comparable with those obtained from primary biomass using conventional methods. The residual pulp of *Gracilaria verrucosa* biomass leftover after agar extraction was reported to have about 40% cellulose (Kumar et al., 2013). In the integrated process, as much as 30% cellulose on a dry weight basis has been recovered from the residual biomass of the green seaweed *Ulva fasciata* (Trivedi et al., 2016). More recently, about 45% of cellulose has been recovered from the residual biomass of *sargassum tenerrimum* (Fig. 3B) with minimum chemical inputs and treatments (Baghel et al., 2020). Interestingly, extraction of seaweed components such as pigments, minerals, proteins, and hydrocolloids before the cellulose extraction has not affected the cellulose yields; it instead got densified in residual mass with the recovery of various components in successive steps (Baghel et al., 2020, 2015; Trivedi et al., 2016). The cellulose remained intact in the residual biomass due to its insoluble nature in the water despite pre-treating the biomass in several aqueous solutions for recovering other products. The cellulose extracted from residual seaweed biomass reported having similar characteristic peaks in FTIR analysis than standard Whatman cellulose. Further, 86-89% saccharification efficiency upon enzymatic hydrolysis confirmed the purity and quality of cellulose (Baghel et al., 2015; Trivedi et al., 2016).

Cellulose extraction from such cellulose-dense seaweed residual mass helps minimize the chemical usage and energy expenses in the extraction process compared with cellulose extraction from whole dried primary seaweed biomass. The cellulose contents (0.85-34% DW) of seaweed are not comparable with lignocellulosic biomass, ranging from 25-45% (Lee et al., 2014). However, the cellulose recovered from residual seaweed biomass is well comparable with that of higher plants as it ranges from 27-40% on a DW basis. The cellulose extraction process for the seaweed's whole biomass and residual biomass was compared with the process of cellulose extraction from lignocellulosic biomass paddy straw to describe the number of treatments and inputs needed for extraction (Rezanezhadet al., 2013)(Fig. 3). Paddy is one of the major crops for Asian countries. Further, paddy straw is the primary low-value agricultural waste. Especially in India, it is used to burn in the field after crop harvesting, creating severe air pollution; thus, it is considered a potential alternative cellulose source. However, cellulose extraction from paddy straw is needed high chemical inputs and harsh treatments (Fig. 3C) such as 3% KOH, 10% HCL, NaClO₂ bleaching, 5% NaHSO₄ treatment followed by 17.5% NaOH to remove lignin and other components present in it while compared with seaweed biomass.

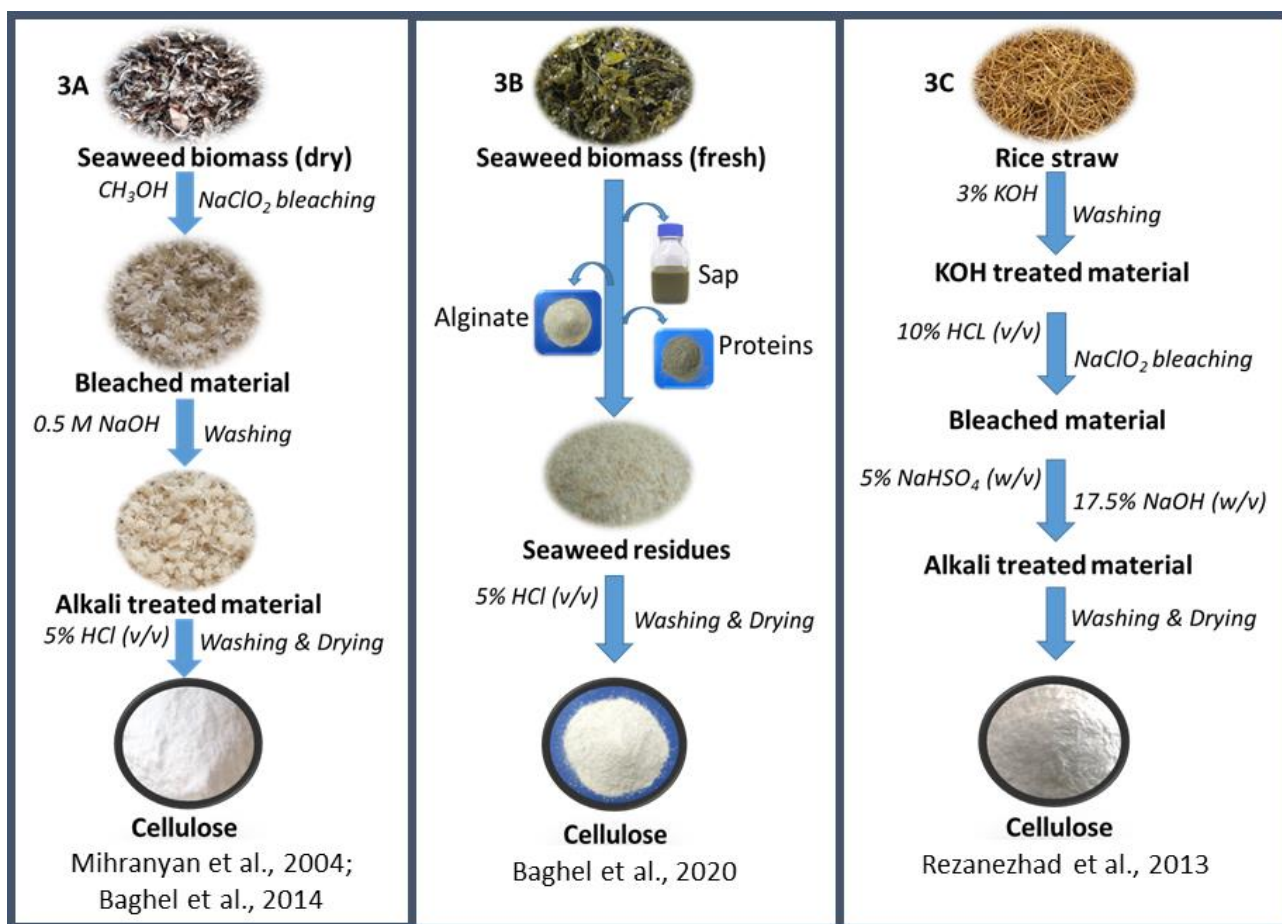


Figure 3 Comparison of cellulose extraction process for seaweed dry biomass; (3A), seaweed residual mass (3B) and rice straw a lignocellulosic biomass (3C).

4. Cellulose from seaweed phycocolloids industrial waste

The global phycocolloids industries consume 5,94,220 dry tons of seaweed biomass annually to produce carrageenan, agar, and alginate (Porse & Rudolph 2017), and residual biomass was treated as waste or without considering for any commercial use (Baghel et al., 2015). These industries mainly using algal species belonging to the group of red and brown seaweeds. Firstly an average cellulose content of 6.31% was calculated using the data published for cellulose contents in several red and brown seaweeds (Table 2 & 3) to estimate cellulose quantity present in annually generated seaweed residual biomass from hydrocolloid industries. Further, based on average cellulose content data, it was estimated that about 37,495 tons of cellulose could be produced annually from residue leftover from hydrocolloid industries using the formula presented as equation 1. The processing of such industrial waste provides cellulose for various applications and helps to convert waste to wealth.

$$ECQ = \frac{AUB \times ACC}{100} \quad (1)$$

Wherein ECQ is Estimated cellulose quantity in hydrocolloids industrial waste (tons); AUB is Annually utilized biomass in tons, and ACC is Average cellulose content reported for red and brown seaweeds (Table 2 & 3).

5. Seaweed-cellulose based products

Although seaweed cellulose research has begun long ago, there are still limited research reports available on its application. Seaweed-based cellulose was widely studied for bioethanol production apart from some scanty reports for its uses in the preparation of the paper, nanocelluloses, microcrystalline cellulose, carboxymethyl cellulose, and bioplastic, etc. The seaweed cellulose-based products are described below in detail.

5.1. Nanocellulose

Nano-celluloses are unique and promising natural materials among the products derived from cellulose. The nanocelluloses are categorized into three types based on structural properties and source; (1) nanocrystalline cellulose (CNC), (2) cellulose nanofiber (CNF) and, (3) bacterial cellulose (BC), also called microbial cellulose (Jawaid et al., 2017). The size and crystallinity are the major characteristics of nanocelluloses. Nanocelluloses are prepared by hydrolysis of amorphous cellulose regions using sulphuric acid (Habibi et al., 2010). The nanocellulose has a typical diameter of 10-50 nm and length in nm to several micrometers (Abe et al., 2007; Jonoobi et al., 2012). The physical and chemical properties of nanocellulose and unique behavior such as liquid crystals, membranes, gel, and emulsions at the nanoscale developed an opportunity for its potential application as a soft material in food, cosmetics, and biomedical applications (Kim et al., 2019; Nystrom et al., 2018; Fatona et al., 2018; Lin & Dufresne, 2014). Nanocellulose is also used in other sectors such as paper and pulp, sensors, and electronics usages. The present estimated global nanocellulose market is USD 297 million and is projected to reach USD 783 million by 2025, with a 21.3% compound annual growth rate (www.marketsandmarkets.com).

There has been extensive research done on the preparation of nanocellulose from different lignocellulosic biomass of terrestrial origin. Mainly pulps such as hardwood pulp, softwood kraft pulp, recycled pulp, and different fibers like cotton fiber, flax fiber, grass fiber, ramie fiber, sisal fiber are used to prepare it (Bettaieb et al., 2015). Last decade few reports were published for nanocellulose preparation either using seaweed biomass or residual seaweed waste. The cellulose nanofibrils were prepared from cellulose extracted from green seaweed *Cladophora glomerata* using a two-step treatment. In the first

step, cellulose was enzymatically hydrolyzed and in 2nd step subjected to mechanical nanofibrillation using a domestic blender at 20000 rpm (Xiang et al., 2016). The prepared nanofibrils' size was 10-40 nm diameter and having a 96.5% crystalline index. Moreover, cellulose nanofibrils showed higher thermal stability in comparison to CNFs produced from bleached eucalyptus pulp (Xiang et al., 2016). The higher thermal stability of CNFs may be attributed to their high crystallinity, low bound water, and high purity. Later various studies have focused on investigating the seaweed-based cellulose for its suitability for nanocellulose preparation (Table 4). There were two reports wherein the researchers successfully prepared cellulose nanocrystals from the residual waste of red and brown seaweed biomass (Achaby et al., 2018; Liu et al., 2017a). Liu et al. (2017a) used kelp waste to prepare NCC with a 52.3 % yield and 69.4% crystallinity index. Achaby et al. (2018) prepared nanocrystal from red seaweed residues leftover after agar extraction by H₂SO₄ hydrolysis for different periods such as 30, 40, and 80 min. The NCC yields and crystallinity index was recorded in the range of 11.18 to 13.76 % and 81 to 87%, respectively. The 80 min acidic hydrolysis yielded 11.18% nanocellulose with 87% crystallinity and a diameter of 5.2±2.9 nm (Fig. 4A). Nanocellulose prepared from seaweed waste was superior in yield, crystallinity, and size to that extracted from whole *Gelidium elegans* biomass (Chen et al.; 2016) (Fig. 4B).

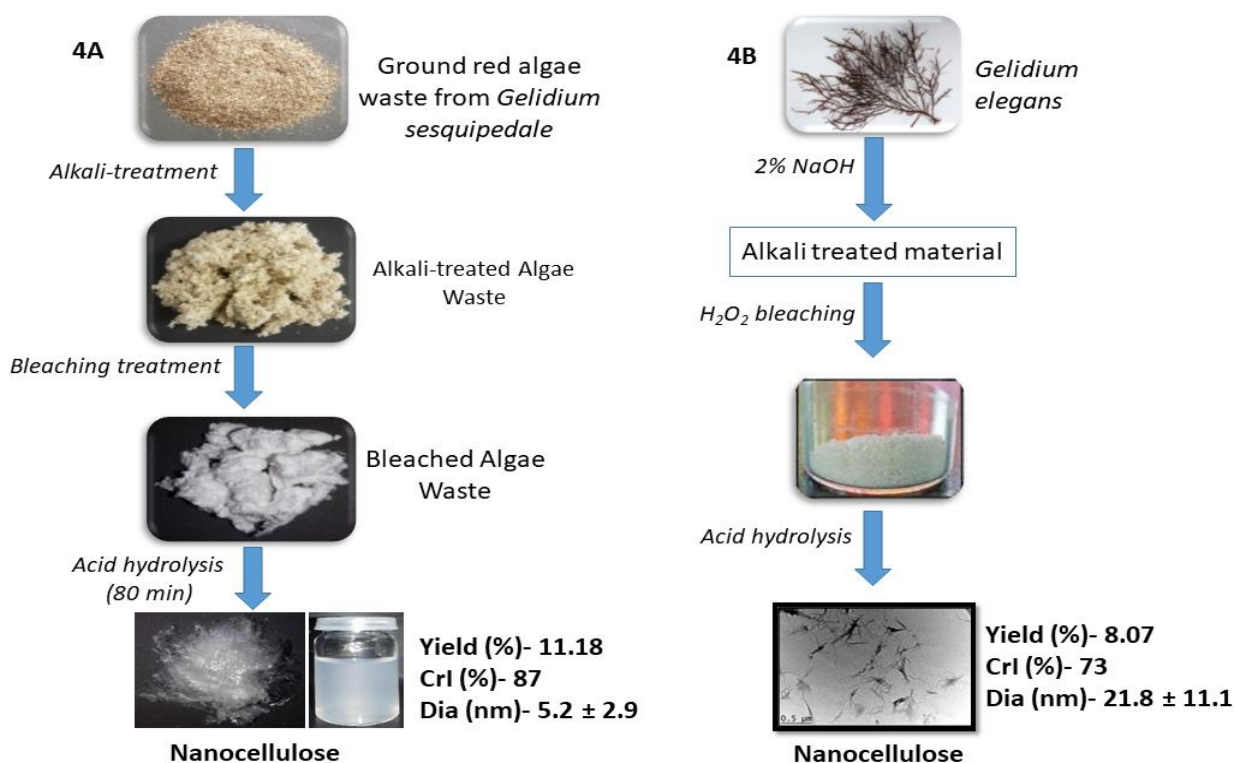


Figure 4 Comparison of nanocellulose isolated from *Gelidium* residual waste left after agar extraction (4A) and whole *Gelidium* biomass (4B). (Fig. 4A adapted and modified from Achaby et al., 2018; Fig. 4B adapted and modified from Chen et al., 2016)

Recently, Doh et al. (2020a,b) studied cellulose from many seaweed species for nanocrystal preparation. The mechanical and chemical treatments were used to prepare nanocrystals having 58-99% crystallinity index and a length ranging 21-248 nm and width 4.8-41 nm analyzed using a high-resolution transmission electron microscope (TEM) and measured with Image J software. Overall, nanocellulose reported from different macroalgal biomass showed a good crystallinity index ranging from 58-99% and size in diameter ranging from 2.3-40 nm (Table 4). There were three different treatments, chemical, enzymatic, and mechanical, alone or in combination, used to prepare nanocellulose from seaweed biomass. The properties of nanocellulose were recorded highly variable with source seaweed species. Thus, it is challenging to predict and recommend the best among these three methods for all seaweeds.

Table 4 Nanocelluloses prepared from different seaweeds

S. N.	Seaweed Species	Product	Treatments	Crystallinity Index (%)	Size, Diameter (nm)	References
1	<i>Cladophora glomerata</i>	Cellulose nanofibrils	Mechanical and enzymatic	96.5	10-40	Xiang et al., 2016
2	<i>Gelidium elegans</i>	Nanocellulose	Chemical treatments	73	17.7-39.9	Chen et al., 2016
3	<i>Laminaria japonica</i> waste	Nanocrystalline cellulose	Chemical treatments	69.4	20-45	Liu et al., 2017a
4	<i>Gelidiella acerosa</i>	Cellulose nanocrystals	Mechanical and chemical	60	32	Singh et al., 2017
5	Red algae waste	Cellulose nanocrystals	Chemical treatments	81-87	2.3-12.2	Achaby et al., 2018
6	<i>Chaetomorpha antennina</i>	Cellulose nanofiber	Chemical treatment	85.02	30	Bhutiya et al., 2018
7	<i>Sargassum fluitans</i> , <i>Laminaria japonica</i> ,	Cellulose nanocrystals	Mechanical and chemical	58-79	4.8-41 nm width, 21-248 nm length	Doh et al., 2020a

	<i>Palmaria</i>					
	<i>palmate,</i>					
	<i>Porphyra</i>					
	<i>umbilicalis,</i>					
	<i>Ulva lactuca</i>					
8	<i>Ulva lactuca</i>	cellulose nanofibrils	Mechanical and chemical	63	--	Wahlstroö m et al., 2020
9	<i>Laminaria</i> <i>japonica,</i> <i>Sargassum</i> <i>natans</i>	cellulose nanocrystals	Chemical treatment	85-99	Length 49-228, width 8- 21 nm	Doh et al., 2020b

To analysis, the suitability of nanocellulose prepared from seaweed-based cellulose for various industrial applications, a comparison was made with the nanocelluloses produced from different lignocellulosic biomass (Bamboo, Rice straw, and Eucalyptus) (Table 5). It was found that nanocelluloses prepared from seaweed-based cellulose are higher crystallinity indexes (58-99%) and comparable sizes (5-45 nm diameter) with those prepared from different lignocellulosic biomass (Table 5). Nanocellulose, prepared from terrestrial biomass, is used widely in biomedical applications such as drug delivery, tissue engineering, cardiovascular applications, wound dressings, cartilage replacements, and medical implants (Guise & Fangueiro, 2016). Uses of nanocellulose-based polymer hybrids in biomedical engineering and water purification are the emerging applications, where nanocellulose plays a vital role as a reinforcing agent to improve materials' native properties (Patel et al., 2019). A few reports have investigated the biomedical application of seaweed-based nanocellulose, especially from *Cladophora* (Bacakova et al., 2019). Liu et al. (2017b) investigated the levels of various impurities present in *Cladophora* based nanocellulose to test its applicability for biomedical applications and found it suitable for future biomedical applications. Sulfonated *Cladophora* nanocellulose bead was prepared and tested for hemocompatibility (Rocha et al., 2018) and its adsorption capacity for Congo Red dye (Ruan et al., 2018). Apart from that, *Cladophora* based nanocellulose was also evaluated for its application as scaffolds for cell cultivation (Mitreveli et al., 2014). Other than *Cladophora* based nanocellulose, nanocellulose prepared from *Cystoseria myrica* combined with Fe₃O₄ was studied to remove mercury ion pollution (Zarei et al., 2018). However, there is more investigation needed to identify the potentials nanocellulose from different algal species.

Table 5 Comparison of nanocelluloses prepared from seaweed-based cellulose and lignocellulosic biomass

Biomass				
Characteristics	Seaweeds	Rice straw	Bamboo	Eucalyptus
Crystallinity Index (%)	58-99	62-76	52-70	34-60
Size in diameter (nm)	5-45	5-35	20-80	7.5-87
References	As per table 4	Thakur et al., 2020; Rezanezhad et al. 2013	Lu et al., 2018; Hu et al., 2014	Qing et al., 2013

5.2. Microcrystalline cellulose (MCC)

Microcrystalline cellulose, as revealed from its name, structurally, is long and thin fibers with a three-dimensional network. Microcrystalline cellulose was discovered in 1955 by Battista and Smith and commercialized under the brand name Avicel1 (Reier, 2013). Microcrystalline cellulose is produced from cellulose with enzymatic or chemical pretreatment followed by mechanical treatment. Hydrochloric acid is mostly used for the hydrolysis of cellulose. MCC is widely used in various industries as a stabilizer, emulsifier, anticaking, and dispersing agent. The estimated global market value of microcrystalline cellulose (MCC) was USD 885.1 million in 2018 and is expected to reach USD 1,241.4 million by 2023, with a 7% compound annual growth rate (www.marketsandmarkets.com). Several reports emphasized the preparation of microcrystalline cellulose from cellulosic material obtained from lignocellulosic biomass (Beroual et al., 2021; Katakajwala & Mohan, 2020; Zhao et al., 2018; Thoorens et al., 2014). Recently, a chemical–mechanical approach was used to prepare microcrystalline cellulose (MCC) from cellulose extracted from brown seaweed *Saccharina japonica* (He et al., 2018). Cellulose was extracted from kelp treated with hydrochloric acid and neutralized, followed by freeze-drying and grinding using a ball mill with ZrO₂ balls (1 cm diameter). MCC produced from *Saccharina japonica* showed high crystallinity index (CrI) of 88.6%. The physical and chemical properties of prepared MCC were comparable with commercial MCC prepared from a higher plant. However, more investigation is needed to explore the suitability of the seaweed biomass to prepare microcrystalline cellulose for various commercial applications.

5.3. Carboxymethylcellulose (CMC)

Carboxymethylcellulose (CMC) is prepared from cellulose by adding carboxymethyl groups (–CH₂-COOH) with some of the hydroxyl groups of the glucopyranose monomers of the cellulose backbone (Heinze & Pfeiffer, 1999). The production of CMC comprises two steps, alkalization and carboxymethylation, using chloroacetic acid serially (Lakshmi et al., 2017). CMC's physicochemical and biological properties make it useful in various industrial sectors such as food, feed, pharmaceuticals, agricultural and wastewater treatment (Ahmad et al., 2014; Borůvková & Wiener, 2013). Mainly, CMC has been prepared from the cellulose extracted from terrestrial biomass. However, recently to find an alternate renewable feedstock, a successful attempt was made to prepare CMC from cellulose isolated from green seaweed *Ulva fasciata* (Lakshmi et al., 2017). The properties of synthesized CMC, like molecular weight (MW) polydispersity and transparency, were comparable with commercial CMC. Degree of Substitution (DS) 0.51 of algal cellulose-derived CMC was found higher than CMC from Pomelo peel with 0.54 (Chumee & Seeburin, 2014) and sugarcane with 0.43 (Hong, 2013). Further, CMC was used to prepare a film with a combination of Ag and leaf extract of *Azadirachta indica* and proved its suitability for the film preparation. However, more detailed investigations from other seaweed species are required to evaluate seaweed-based cellulose's potentials to prepare CMC and its commercial applications.

5.4. Paper preparation

The production of paper and paperboard is the primary non-food application of cellulosic pulp. World production of paper and pulp was estimated at 419.72 million metric tons in 2018, and market value projected to reach 523.6 billion U.S. dollars in 2022 (www.statista.com). Commercially cellulose is obtained from wood pulp, and cotton is mainly used to manufacture paperboard and paper (Bogati, 2011). Seaweed cellulose is projected as a raw material for paper-making as an alternative to wood-based cellulose as seaweed can produce a larger mass of material in a shorter time than terrestrial-based plants. It is also assumed that the use of seaweed cellulose for paper production will help overcome environmental issues like deforestation and global warming (Mukherjee & Keshri, 2018).

Curtis et al. (1924), for the first time, patented the process for the preparation of pulp utilizing the cellulose and alginate present in fresh alginophytic seaweed. Pulp comprises of the finely divided cellulose having its fibrous structure substantially destroyed suspended in a solution of alginate and proposed its utilization for binding or agglomerating agent. Further, a process was patented to prepare paper from seaweed in combination with wood pulp (Nicolucci & Monegato, 1995). About 8-12 wt.% of seaweed particles ($\leq 500 \mu\text{m}$) prepared from the green seaweed *Ulva rigida* and *Ulva lactuca* mixed with bleached wood-pulp and calcium carbonate fed into a paper-making machine. The use of algal material improved

the mechanical (resistance to bursting, stiffness, and rupture length) and chemical characteristics of the paper. The paper was recently prepared using bleached pulp produced from two red algae species, *Gelidium corneum*, *Gelidium amansii*, and paper properties compared with bleached wood chemical pulps-based paper (Seo et al., 2010b). The paper prepared from red algae pulp was found superior to wood pulp-based paper in high Bekk smoothness and opacity, which are essential properties for quality printing paper. The commercial production of quality papers from macroalgal cellulose has a long way to go, and it needs a high volume of cellulose and extensive investigation.

5.5. Bioplastic

Polymers possess a wide range of properties and became a part of everyday life (Cheng, 2008). The polymers are classified into two categories -synthetic and biopolymers. Plastic is one of the best examples of synthetic polymers which have an important role in everyday human life globally. Synthetic polymers are made up of carbon-carbon bonds as their backbone, typically derivatized in a controlled environment from petroleum oil (Shrivastava, 2018). Properties of synthetic polymers such as strength, high elasticity, resistance to chemical, and physical degradation make them unique and useful (Bhadra et al., 2014). However, these polymers' non-degradable nature has become a severe environmental crisis (Kyrikou & Briassoulis, 2007). The disposal of synthetic plastic waste becomes a major challenge for the world, and now attention is turning towards biopolymers. The properties like renewability, biodegradability, and biocompatibility are the major characteristics of biopolymers-based plastic making it suitable for several industrial and medical applications (Dassanayake et al., 2018). However, bioplastics' high water absorption tendency is one of the potential drawbacks as it may alter the resulting plastic's elastic modulus, thus making plastic with a lower elasticity. Similarly, the low thermal stability of bioplastics limits its applicability.

Production of bioplastic is one of the emerging applications of seaweeds and their cellulose. The bioplastics were synthesized using sorghum and red seaweed *Eucheuma spinosum* fiber (Darni et al., 2019). The properties of prepared bioplastic such as tensile strength (21.265 MPa), elongation (4.467%), Young's Modulus (498.463 MPa), density (0.95 g/cm³), and water uptake (21.265%) was comparable with characteristics of commercial HDPE plastic. Recently, nanocomposite films were prepared using crude extracts and cellulose nanocrystals (CNCs) isolated from two seaweeds, *Laminaria japonica*, and *Sargassum natans*, with suggested applications in food preservation or to design functional foods (Doh, 2020c). The uses of cellulose nanocrystals (CNCs) improved the physicochemical, mechanical, barrier, and thermal properties of biofilms. Similarly, Kamarol Arifin (2020) developed biofilm using a combination of cellulose extracted from a green seaweed *Caulerpa lentillifera* and corn starch that showed good biodegradable properties. The bioplastic film prepared with 10% cellulose concentration showed the

highest tensile strength and increased Young Modulus value. Overall, studies on the uses of seaweed-based cellulose to prepare bioplastics confirmed that it could be used as a filler in producing bioplastics.

5.6. Production of cellulosic ethanol

The non-renewable nature of petroleum-based fuels and the negative consequences on the environment of their usage has given rise to find an appropriate and efficient alternative. Among the possible alternative to petroleum fuels, bioethanol production is the primary target globally. Most ethanol produced is first-generation bioethanol which comes from plant-derived sugar such as sugarcane, sugar beet, corn, and wheat. Due to the rising concern over the “Food vs. Fuel”; the lignocellulosic materials for bioethanol production got highlighted (Baghel et al., 2015). However, removing lignin from such biomass before hydrolysis is a major challenge affecting the economics of bioethanol production and generating chemical effluents (Trivedi et al., 2013). After first and second-generation resources, seaweed biomass emerged as a third-generation resource for bioethanol production (Kumar et al., 2013).

Seaweeds constitute high carbohydrate, low lignin content, and a higher productivity without any agricultural inputs. Firstly, bioethanol was produced from the extract of brown seaweed *Laminaria hyperborean* (Horn et al., 2000). Afterward, various researchers reported the potential of different seaweeds for bioethanol production (Lee & Lee, 2012; Ge et al., 2011; Adams et al., 2009; Uchida & Murata, 2004). Although there were many developments in seaweed-based bioethanol research, the challenge remains to achieve high ethanol yields. The chronology for the production of bioethanol from seaweed biomass is presented in Figure 5. As only cellulose fraction of seaweed biomass, which is in minor proportion, is easily hydrolyzed and fermented while a larger part of pentose type of sugar is difficult to hydrolysis and ferment using common routes. Later, an engineered microbial platform for direct bioethanol production using either alginate alone (Takeda et al., 2011) or whole biomass (Enquist-Newman et al., 2014; Wargackiet al., 2012) has also been developed to utilize the maximum carbohydrates fraction. However, a concern was developed that utilization of whole polysaccharides of seaweed whole polysaccharides for bioethanol production could not sustain for a long time (Baghel et al., 2015). Polysaccharides such as agar, carrageenan, and alginate extracted from seaweed biomass are high-value products compared to bioethanol and have established a global market. The commercial uses of such polysaccharides fraction for bioethanol in the long term may jeopardize the multibillion seaweed hydrocolloid industries. Such seaweed biomass could be utilized for bioethanol production in the case of surplus biomass production only. Subsequently, the attention has been shifted towards the production of ethanol from only seaweed cellulose fraction in biorefinery approaches. The extraction of cellulose from 3 different agar-producing seaweeds after recovery of pigments, mineral, lipid, and agar was reported, and further cellulose was hydrolyzed and fermented to produce bioethanol (Baghel et al., 2015). Similarly,

bioethanol was produced from cellulose present in residual waste of green seaweed *Ulva fasciata* and red seaweed *Gracilaria corticata* (Trivedi et al., 2016; Baghel et al., 2016).

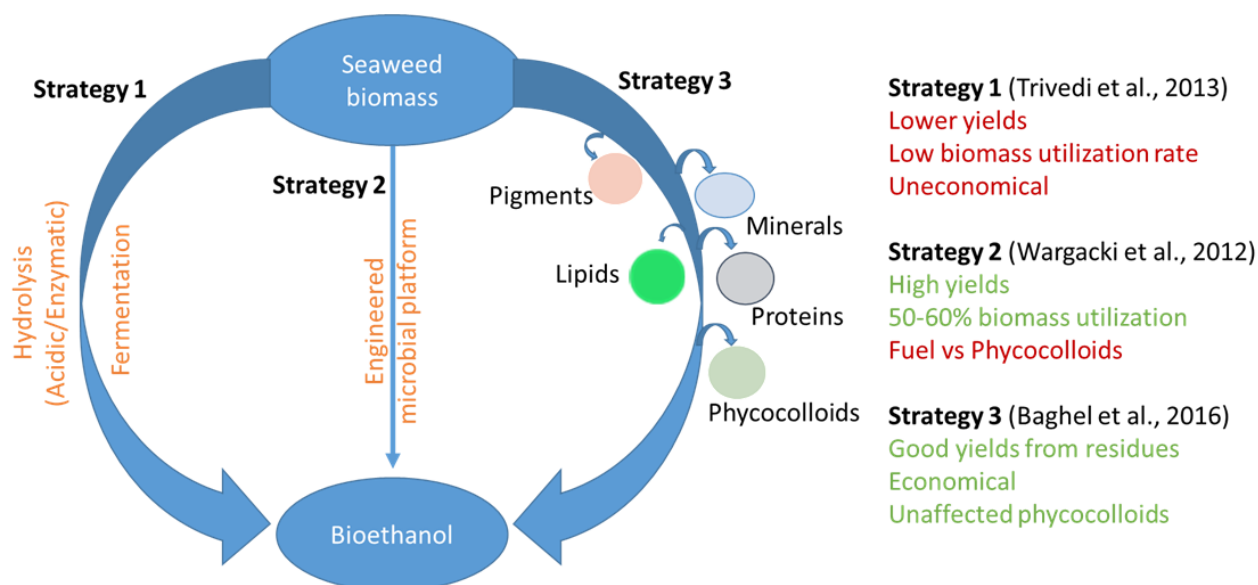


Figure 5 Strategies for bioethanol production from seaweeds

6. Conclusion and future perspectives

Cellulose extracted from various seaweed species has shown similar properties to those produced from lignocellulosic resources. The lower content of cellulose in seaweed biomass is a major drawback; negligible or no lignin content is beneficial. The seaweed cellulose-based products could be used in various industrial applications, as proved in various research reports. There is an immense opportunity to extract seaweed cellulose and their application development with the growing seaweed global production. Based on a detailed analysis of various research papers published on seaweed cellulose, it can be inferred that (1) The yields of cellulose for most seaweed species are not comparable with those from lignocellulosic biomass. (2) The cellulose yields (27-45%) from residual seaweed waste are comparable with that of lignocellulosic biomass and need efforts to utilize such waste to produce cellulose and create value from the waste. (3) It could be feasible and economical to extract cellulose from seaweed biomass as a co-product. (4) There is a need for technological inventions for large-scale farming of cellulose-rich algal species such as *Cladophora*, *Ulva*, *Gelidiella*, and *Gelidium* for cellulose production. (5) Since demand increases for cellulose-based soft materials, using seaweed-based cellulose to prepare high-value products such as nanocellulose and their application as soft material in food and cosmetics could be a topic of future research.

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