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Review

A Brief Review on the Development of Alginate Extraction Process and Its Sustainability

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Abstract: Alginate is an attractive marine resource-based biopolymer, which has been widely used in pharmaceutical, food and textile industries. This paper reviewed the latest development of the conventional and alternative processes for alginate extraction from brown seaweed. To improve extraction yield and product quality, various studies have been carried out to optimize the operation condition. Based on literature survey, the most commonly used protocol is soaking milled seaweed in 2% (*w/v*) formaldehyde, overnight, solid loading ratio of 1:10–20 (dry weight biomass to solution), then collecting the solid for acid pre-treatment with HCl 0.2–2% (*w/v*), 40–60 °C, 1:10–30 ratio for 2–4 h. Next, the solid residue from the acid pre-treatment is extracted using Na_2CO_3 at 2–4% (*w/v*), 40–60 °C, 2–3 h, 1:10–30 ratio. Then the liquid portion is precipitated by ethanol (95%+) with a ratio of 1:1 (*v/v*). Finally, the solid output is dried in oven at 50–60 °C. Novel extraction methods using ultrasound, microwave, enzymes and extrusion improved the extraction yield and alginate properties, but the financial benefits have not been fully justified yet. To improve the sustainable production of alginate, it is required to promote seaweed cultivation, reduce water footprint, decrease organic solvent usage and co-produce alginate with other value-added products.

Keywords: sodium alginate; brown seaweed; Phaeophyceae; green processing; alginic acid

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1. Introduction

Alginate was firstly described by a British chemist E.C.C. Stanford in 1881 [1], as a naturally occurring polysaccharide isolated from brown seaweed. Recent data published by the United Nations—Food and Agriculture Organization (FAO) showed that approximately 34.5 million tons of seaweeds (brown, green and red) was produced worldwide in 2019. Among it, red and brown seaweed accounted for 52% and 47%, respectively, whereas green seaweed only contributed to 0.04% of the overall production [2]. Brown seaweed is a renewable biomass due to its abundant availability around the world, and the seaweed based bio-production process is considered biodegradable, biocompatible and environment-friendly [3,4].

Alginate is abundant in brown seaweed (Phaeophyceae) consisting of up to 40% (dry matter) [5]. Brown seaweed is cultivated in both northern and southern hemispheres mainly in Australia, Chile, China, Denmark, Spain, France, Indonesia, India, Ireland, UK, US and South Africa. Industrial-scale alginate production commenced in 1929 using it as a boiler additive, can-sealing compound and later as a food additive such as ice-cream stabilizer [6]. It is estimated at least 30,000 metric tons of commercial alginate are produced globally per annum. The major alginate producers are concentrated in six countries: China, USA, UK, Japan, Chile and Germany [7,8]. Alginate market value is predicted to reach $1.07 billion by 2028 and a Compound Annual Growth Rate (CAGR) of 5.0% [9]. Worldwide alginate production is predominantly derived from brown seaweed, such as genera *Laminaria*, *Macrocystis* and *Ascophyllum* [10]. A significant amount of alginate is harvested from wild seaweed with exception of China, where aquaculture is used as the source for alginate [11].

Besides seaweed, alginate can also be extracted from bacteria species such as *Azotobacter* spp. and *Pseudomonas* spp. [12]. *Laminaria*, *Microcystis* and *Ascophyllum* are the only three types of brown seaweed that are deemed to be sufficiently in abundance or suitable for commercial use for alginate extraction. Other seaweed species such as *Sargassum* are typically used only when there is a shortage of commercial sources [7]. This is due to alginate recovered from *Sargassum* been considered to be of "borderline" quality [7].

Alginate is a natural occurring biopolymer with complex matrix polysaccharides in the brown seaweed. It exists as an insoluble salt such as calcium, magnesium and sodium [13]. Commercially available sodium alginate's molecular weight range between 32–400 kDa [4]. Alginate consists of a linear block copolymer sequence of α-L-guluronic acid (G) and β-D-mannuronic acid (M) bonded by 1→4 linkages as shown in Figure 1. The difference at C-5 in the uronic acids makes guluronic acid and mannuronic acid stereochemically different to each other Figure 1c. Random sequences of M- and G-blocks are distributed, where the proportion of these sequences depends on the source [14]. The physical properties of alginate depend on a number of different key factors in which M and G contents play a crucial part. The M-M block is a relatively straight polymer, linked di-equatorially at C-1 and C-4; whereas the G-G block is buckled, linked from di-axial groups at both C-1 and C-4 [15]. Figure 1c shows a stereochemical structure of M-G, G-G, G-M, M-M blocks. Around 200 different categories of alginates have been identified and extracted from nature, the ratio of mannuronate to guluronate (M/G) varies significantly, depending on the source organism and tissue from which it is being isolated and also the season when it was harvested [16]. Table 1 listed the M/G ratios of alginates extracted from typical species of brown seaweed.

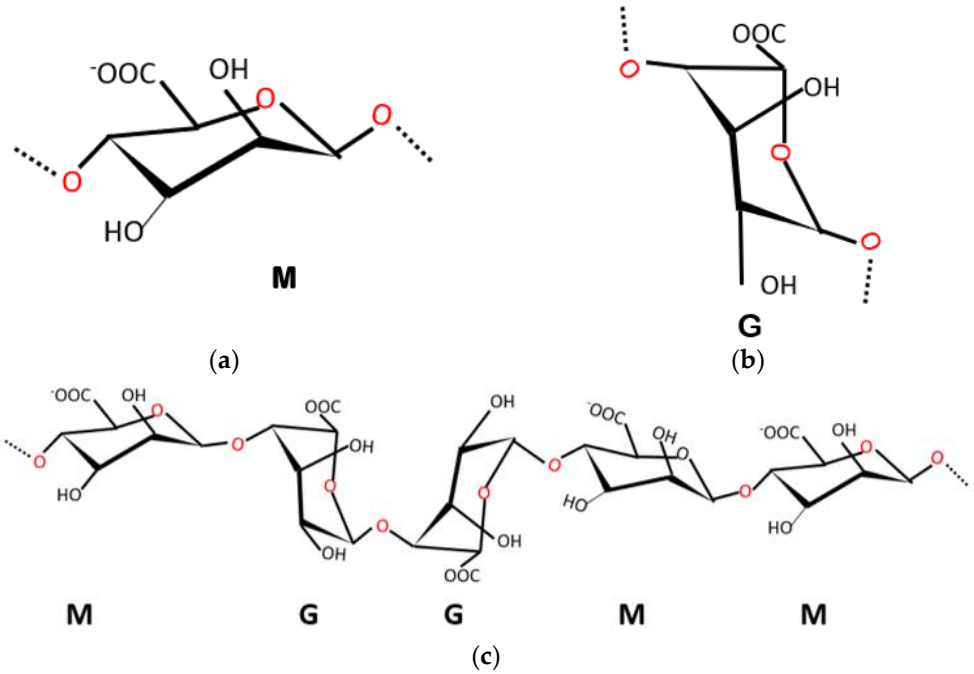

Figure 1. Polysaccharides chemical structure (**a**) β-D-mannuronic acid (**b**) α-L-guluronic acid, (**c**) M and G block conformation of alginate.

Table 1. The M/G ratio of alginate extracted from various seaweed species.

Species	Mannuronic Acid (%)	Guluronic Acid (%)	M/G Ratio	Reference
Ascophyllum nodosum	46.0	54.0	0.85	[17]
Bifurcaria bifurcata	65.0	35.0	1.88	[18]
Fucus guiryi	82.0	18.0	4.41	[18]
Fucus vesiculosus	59.0	41.0	1.44	[17]
Laminaria digitata	59.0	41.0	1.44	[19,20]
Laminaria hyperborea, fronds	56.0	44.0	1.28	[21]
Laminaria hyperborea, stems	30.0	70.0	0.43	[21]
Laminaria japonica	65.0	35.0	1.86	[22]
Laminaria ochroleuca	72.0	28.0	2.52	[18]
Macrocystis pyrifera	61.0	39.0	1.56	[23]
Saccharina longicruris	41.0	59.0	0.69	[17]
Saccorhiza polyschides	63.0	37.0	1.73	[18]
Sargassum vulgare	56.0	44.0	1.27	[24]

2. Alginate Production Process—A Conventional Approach

Extraction of alginate from seaweed is a multi-stage process. Figure 2 shows a simplified schematic diagram of conventional alginate production routes, which are thoroughly discussed in the following sections with the support of published scientific papers and patents. Briefly, fresh seaweed is firstly washed, dried and milled into powder. Then, the seaweed biomass is soaked in water to rehydrate, to which various chemicals are added to remove un-wanted compounds in the seaweed. Then, an acid or alkali pre-treatment is applied to break the plant cell wall, which is followed by sodium carbonate extraction to obtain water-soluble alginate from the seaweed biomass matrix. There are three precipitation routes to recover alginate from the solution, namely sodium alginate route, calcium alginate route and alginic acid route, with the final product usually isolated in the form of sodium alginate.

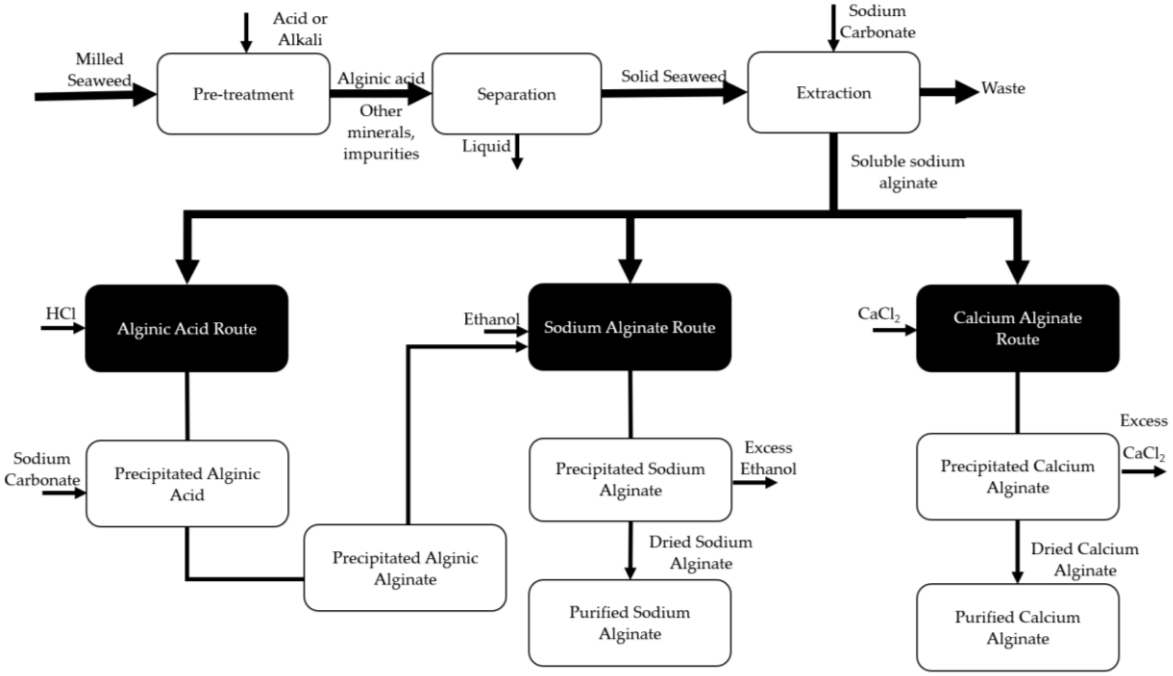

Figure 2. Schematic diagram of conventional alginate extraction processes.

2.1. Pretreatment

2.1.1. Mechanical Treatment

Mechanical size reduction of seaweed is the first step in the extraction process of alginate. After harvest, dried seaweed biomass is cut into small pieces to enable processing. In most processes, the dried seaweed is further milled/ground into powders with a particle size of around 0.5 mm. It is expected that the finer particles have a higher surface area, which leads to an increase in contact with the solvents/chemicals used during the pre-treatment/extraction stages and thus increases the extraction efficiency. Particularly, Fertah et al., 2017 investigated the impact of seaweed particle size on the alginate extraction yield [17]. Alginate extractions using a *Laminaria digitata* sample with particle sizes of less than 1 mm and a sample with particle sizes in the range of 1–5 mm, were carried out. Higher alginate extraction yields were obtained when seaweed with smaller particle size were used in the extraction at three different temperatures. Particularly, at 40 °C, the alginate extraction yield increased from 44.0% (seaweed particle size 1–5 mm) to 51.8% (seaweed particle size <1 mm). Although the method of calculating alginate yield was not reported in the paper and the purity of the product was not known, a direct comparison of two experiments demonstrated the benefits of using a finer particle size. Seaweed with a particle size of 0.25–1 mm is widely used in lab-based research [18–20], as shown in Table 2. Further reducing the particle size could possibly increase the extract yield, but higher energy consumption could be encountered, which may make the process economically unfavourable.

2.1.2. Soaking

Soaking of milled seaweed powder in 2% (*v/v*) formaldehyde is widely used in around half of the alginate extraction processes reported in the literature (Table 2). Mild temperature of 20–40 °C for up to 24 h is applied to soften the seaweed tissue walls, to remove pigments and to remove phenolic compounds [20,21]. The effect on the intrinsic viscosity of alginate derived from *Sargassum polycystum* seaweed soaking at different formaldehyde concentrations and soaking time at 28 °C was studied by Wedlock et al., 1990 [22]. It was concluded that increase in the concentration of formaldehyde and the soaking time led to high intrinsic viscosity. However, the benefit of soaking over 24 h was significantly reduced. As a result, formaldehyde soaking normally does not exceed 24 h [23,24]. High intrinsic viscosity can be caused by the removal of phenolic or poly-phenolic compounds, which are present in the seaweed matrix. The presence of phenolic compounds during the extraction stage can contribute to the degradation of the polysaccharide, which ultimately reduces the intrinsic viscosity of the alginate [22]. During the soaking process, phenolic compounds react with formaldehyde to produce an insoluble polymeric solution and then are separated from the extraction system. The residue phenolic compounds can be removed at a later stage in the acid pre-treatment. Thus, the extraction solution pH plays a significant role in the intrinsic viscosity. The phenolic compounds present in seaweed vary from species to species, therefore, alginate extracted from different seaweed species could have a significantly different intrinsic viscosity although the same extraction procedure is followed. [22] Besides formaldehyde, alternative chemicals can be used at the soaking stage to facilitate the removal of pigmentation, fat, protein and the phenolic compounds from the seaweed, such as ethanol [20], acetone [25], sodium hypophosphite [26], chloroform [27] and petroleum ether [28]. Soaking using only water is also reported in several processes [29], which is mainly to rehydrate and swell the dried seaweed particles.

2.1.3. Acid and Alkali Pre-Treatment

Acid pre-treatment is one of the first methods to be implemented by early inventors such as the method developed by Clark and Green in 1930 [30]. Dilute acids, mainly HCl, have been utilized to remove non-targeted compounds present in the seaweed such as polyphenols and easily degradable polysaccharides (e.g., fucoidans) [31]. Furthermore, dilute acid also eliminates polyvalent cations, such as Ca^{2+} and Mg^{2+} from the alginate

matrix by converting alginate from the salt form into alginic acid [7]. Myklestad [32] demonstrated that ion-exchange during acidic pre-treatment followed a first-order reaction and the exchange rate is proportional to the logarithm of acid concentration in the solution. In the acid pre-treatment stage, the pH needs to be adjusted to be lower than 4 for the efficient removal of polyvalent cations. [11] Typical acid pre-treatment is carried out for 30 min–24 h, depending on the temperature and acid concentration (Table 2). The concentration of acid ranged from 0.1–5.5 M with pH ranging from 1.8–2. One example is the procedure reported by Trica et al., 2019, in which 0.1 M HCl was used at 60 °C for 2 h with a biomass loading ratio of 1:20 (*w/v*, dry weight seaweed biomass to acid solution) [20]. The impact of acid pre-treatment on the alginate extraction yield, molecular weight and co-production of fucoidan was investigated by Lorbeer et al., 2015 using a response surface method [33]. The initial acid pre-treatment was carried out at 25, 35 and 45 °C, at pH 2, 3.5 and 5 adjusted by HCl; with extraction time of 10, 60 and 110 min. It was found that all three parameters have a significant impact on the extraction yield, while the composition of alginate (M/G ratio) did not change significantly. Using the parameters predicted by the model developed from the initial experiment, alginate extraction yields up to 45.4% were obtained at pH 1, 55 °C, extracting for 160 min. The molecular weight of the alginate was up to 986 kDa, which is one of the highest alginate molecular weights reported in literature. The high molecular weight could relate to the seaweed species (*Ecklonia radiate*), as a similar acid pre-treatment with 0.2 M HCl, 60 °C, extracting of *Nizimuddinia zanardini* for 180 min only led to an alginate sample with a molecular weight of 103–119 kDa [24]. Organic acids, such as citric acid, have been recently explored for pre-treatment of seaweed as a renewable alternative to inorganic acid. Fawzy and Gomaa 2021 reported a process using citric acid as a pre-treatment solution to sequentially extract fucoidan and alginate from *Sargassum latifolium*, [25]. Similar to Lorbeer et al., 2015 [33], a surface response method was used to investigate the impact of citric acid concentration (pH), temperature and pre-treatment time. In this study, the M/G ratio changed significantly at different pre-treatment condition, ranging from 0.34 to 1.05. In two almost identical trails, reducing the citric acid concentration from 3% to 1% (*w/v*) changed M/G ratio from 1.01 to 0.34, which was significant. The molecular weight of the alginate sample was relatively low, in the range of 142–194 kDa with a yield of 21.1–31.8%.

Although alkali pre-treatment of seaweed is less popular than acid pre-treatment, it has been reported in several patents in the 1960s as well as recent research papers [34,35]. The preliminary target for the alkali pre-treatment is to degrade the seaweed cell wall to enable the subsequent alginate extraction. Treating seaweed with boiling alkali is reported in two Russian patents published in 1960s (cited by Hernandez-Carmona et al., 1999 [34]), as well as soaking at room temperature up to 2 h [35]. However, no detailed yield and characterisation of the alginate products were reported.

2.2. Extraction

After pre-treatment, the seaweed biomass is washed with water to neutralize pH. Addition of alkali or acid is also reported if it is needed. Then the solid residue is separated by filtration or centrifugation for the following alginate extraction process. Extraction using sodium carbonate is predominant, in which the insoluble alginate acid and/or alginate salts are converted into soluble sodium alginate. Then, the soluble sodium alginate is released from the seaweed biomass matrix, transporting it into the liquid phase (extraction solution). The extraction efficiency and the alginate property are mainly affected by extraction temperature, time, pH and alkaline concentration [27], which are discussed in detailed in the following sections.

2.2.1. The Impact of Temperature

Typical alginate extraction uses 2% (*w/v*) sodium carbonate at a temperature ranging from 20–100 °C [17,23,25,35,36]. Increase in extraction temperature led to an increased amount of the alginate released from the seaweed biomass matrix, therefore a higher yield.

However, higher temperature enhances the degradation of alginate polysaccharide chain, leading to a lower viscosity alginate product. Fertah et al., 2017 studied the impact of temperature on the alginate extraction yield using *Laminaria digitata* [17]. The extraction was carried out at three different temperatures (25, 40 and 60 °C). The results showed that the highest extraction yield (51.8%) was obtained at 40 °C with seaweed particle size less than 1 mm. Further increase in the temperature leads to a degradation of polymer chains and a lower yield [17]. As shown in Table 2, majority of the extraction processes reported in the literature were carried out at around 40–60 °C. [20,25,36,37] Interestingly, Mazumder et al., 2016 investigated alginate extraction from *Sargassum muticum* at a higher temperature range (50, 60, 70, 80, 90 and 100 °C) [36]. The alginate yield was only 6% at 50 °C, which dramatically increased when the temperature increased to 80 °C. The highest yield of approximately 10% was achieved at 90 °C. The optimum temperature to achieve a higher yield is depended on seaweed species [17,36,38], which is also affected with other extraction parameters, such as extraction time and pH. Furthermore, a higher yield is not always the most desired target for an alginate extraction process; the properties of the alginate product, e.g., viscosity, molecular weight and M/G ratio are sometime crucial for alginate application. Therefore, in alginate extraction the optimization of extraction temperature is commonly carried out together with the optimization of other parameters (Table 2).

2.2.2. The Impact of Time

Extraction time is another critical variable that affect the yield and properties of the alginate. Various extraction time has been used in literature mainly in the range from 2 to 3 h (Table 2). An early patent published in 1937 claimed 20–40 min of extraction was sufficient to extract alginate, in which direct steam heating was used [30]. The investigation carried out by Hernández-Carmona et al., studied the effect of extraction time in the range of 1–9 h on the alginate yield extracted from *Macrocystis pyrifera* [38]. It was concluded that alginate yield reached a maximum at 3.5-hour of extraction (43%) and then reduced. Similarly, a study conducted by Mazumder et al., 2016 identified a direct correlation between extraction time and yield in the alginate extraction of *Sargassum muticum* [36]. It concluded that the yield increased with the extraction time until 3 h, and then reduced. The extraction time also has an impact on the rheological properties of the alginate. Truus et al., 2001 demonstrated that increasing the extraction time and temperature accelerated de-polymerization of the alginate chain, this subsequently resulted in an alginate product with lower viscosity [39]. However, a recent study conducted by Torres et al., 2007 reported an opposite observation [40]. In alginate extraction from *Sargassum vulgare*, a "low viscosity" alginate was recovered after 1-hour extraction at 60 °C; while a "high viscosity" alginate was obtained when the extraction time increased to 5 h. It was explained that increase in extraction time led to high molecular weight polysaccharide dissolute in the alkali solution [40].

2.2.3. The Impact of Extraction Chemical and pH

Sodium alginate is soluble in alkaline solutions. Sodium carbonate (Na_2CO_3) is predominately used in the extraction stage due to its low cost and can easily achieve the desired pH (11 or over) during the extraction stage [36,41]. The direct adjustment of pH in alginate extract is relatively complex, especially in industrial scale operation and the range of pH changes is relatively small (from 11.7 at 0.5% Na_2CO_3 to 12.4 at 5% Na_2CO_3) [36]. Therefore, most research adjusted the Na_2CO_3 concentration to investigate the impact of pH (Table 2). Mazumder et al., 2016 concluded 3% was the optimum Na_2CO_3 concentration in alginate extraction from *Sargassum muticum* in the range of 1–5% (*w/v*) [36]. This fits with other publications as a sodium carbonate concentration of 2–4% is predominantly used in various studies (Table 2). A higher Na_2CO_3 range of 5–10% (*w/v*) was investigated in alginate extraction from *Sargassum natans* [18]. The results showed that 5% was the best in the range investigated while higher Na_2CO_3 concentration resulted in reduced

alginate yield. It was explained that alkali reacted with the β-glycoside bond in alginate and degraded the polysaccharide. As a result, it is recommended that the pH of the extraction system should be controlled to 10 or less. However, in a study extracting alginate from *Sargassum binderi* and *Turbinaria ornate* with the assistance of ultrasound, among 12 trials recommended by fractional experimental design, 3 experiments carried out at a pH of 12 resulted in yields of 33.41–54.06%, while the other 9 experiments (pH 8 or 10) resulted in yields of 0.47–1.64% [42]. Apart from Na_2CO_3, other sodium salts/alkali, such as NaOH [43,44] $NaHCO_3$ [45] and NaCl [43–45] have also been used in alginate extraction, as discussed in the Section 2.2.2. Interestingly, Guo and Zhang 2020 explored the possibility of using a solution containing 3.5% sodium chloride and 28% ammonia to extract alginate [43]. In three conditions in which the pH of the solution was adjusted to 11 by sodium hydroxide, potassium hydroxide and sodium carbonate, respectively, the solution using potassium hydroxide led to the best extraction yield of 40.46% and the highest molecular weight of 256 kDa. This suggested that the ionic strength and composition in the extraction solution could have an impact on the alginate extraction process. For alginate extraction using chemicals other than Na_2CO_3, the extraction time and temperature varies significantly. Davis et al., 2004 explored alginate extraction using HCl at pH 6.5–7.5 and 4% (*w/v*) NaOH, respectively, both operated at 20 °C overnight with a seaweed loading ratio of 1:100 (*w/v*). The alginate yields are comparable with a conventional Na_2CO_3-based extraction process [44] (Table 2). The impact on the M/G ratio is also negligible. A new method using ionic liquid to extract alginate was proposed in a recent patent [46] in which milled seaweed was mixed with 10% choline hydroxide and sodium chloride eutectic solvent to produce alginate. It was operated at 100–130 °C for 1–5 h.

2.3. Alginate Separation and Purification

The extracted sodium alginate dissolves in alkali solution, forming high viscous slurry. To obtain pure alginate, the residue seaweed biomass needs to be separated out by filtration and centrifugation, then the sodium alginate is precipitated via sodium alginate, calcium alginate or alginate acid route. Then, the solid form of alginate is separated out, re-dissolved in alkali solution, and precipitated again to reach required purity.

2.3.1. Separation

Filtration is a commonly used method to separate alginate from the solid seaweed residue. As the slurry viscosity is high, 2–3 folds volume of water is added to dilute the solution to enable efficient filtration [47]. Hernández-Carmona et al., 2013 suggested that the slurry viscosity should be reduce to 45 mPa.s, corresponding to 1:55 initial seaweed solid to liquid ratio [11]. In industrial operation, the slurry containing sodium alginate and waste seaweed biomass is then transferred to a rotary vacuum drum filter, which removes the solid particles efficiently from the sludge, producing dewatered seaweed residue and a clear solution of alginate. In lab research, filter paper [38] and muslin cloth [48] is normally used to remove seaweed biomass. Alternatively, centrifugation can also be used to separate alginate solution.

2.3.2. Precipitation

The alginate precipitation method could be divided into three routes: sodium alginate, calcium alginate and alginic acid routes (Figure 2). The sodium alginate route is the most widely used process, in which an organic solvent, mainly ethanol is added into the sodium alginate solution to reduce the alginate solubility and therefore precipitate sodium alginate (Table 2). The fibre like solid form of sodium alginate is then separated from the solution via filtration or centrifugation. Besides ethanol, other organic solvents, such as isopropanol and acetone can be used. In the calcium alginate route, $CaCl_2$ is added into the sodium alginate solution, converting sodium alginate into insoluble calcium alginate, which can then be separated from the solution. Alginic acid also has a low solubility in water. Conversion of sodium alginate to alginic acid by adding HCl could also precipitate alginate. In both the

calcium alginate and alginic acid routes, the separated solid form of alginate is commonly converted to sodium alginate solution again by reacting with sodium carbonate, and subsequently is precipitated by organic solvent, e.g., ethanol [49,50]. The main objective of applying the calcium alginate or alginic acid route is to reduce the usage of organic solvent by introducing alternative purification pathways. In the alternative purification pathways, organic solvent can be used only once in the final purification step, while the sodium alginate route normally uses organic solvent twice; in the initial precipitation and final purification step. The direct comparison of three precipitation routes was carried out by Gomez et al., 2009 using brown seaweed *Macrocystics pyrifera* [49]. It was concluded that the sodium alginate route not only had the simplest steps, but also resulted in the highest yield with good overall rheological properties. The calcium route led to a product with the lowest molecular weight and poor mechanical properties. It is explained that the degradation of the ether bond by HCl (which is used in both the calcium alginate and alginic acid routes) was responsible for the low molecular weight and weak mechanical properties. In another study comparing the calcium and alginic acid routes using the brown seaweed *Sargassum sp,* the calcium route led to a higher yield and a better product than that of the alginic acid route [51]. However, the comparison of these two routes with the sodium alginate route was not reported and the product was the alginic acid form instead of sodium alginate.

2.3.3. Drying

In lab scale research, alginate samples can be dried overnight in an oven [11], a freeze drier [18] or a vacuum drier [27] at 30–75 °C. For industrial scale production, a fluidize-bed dryer is normally used. After drying, the alginate product is milled by turbine mill or fixed hammer mill to reach the required particle size, e.g., lower than 250 μm [52].

Table 2. Comparison of alginate extraction processes using conventional method.

Seaweed Species	Size	Soaking	Acid/Alkali	Extraction [a]	Precipitation [b]	Drying	Yield (*w*/*w*), mw (kDa), M/G [c]	Ref.
Alaria esculenta; Saccharina latissima; Ascophyllum nodosum	0.5 mm		0.2 M HCl 24 h, (ratio 1:25 *w*/*v*)	0.1 M $NaHCO_3$ 2 h (ratio 1:45 *w*/*v*) & NaOH pH 8	Isopropanol	65 °C overnight	14.6–38.6%, -, -	[45]
Ascophyllum nodosum	0.25 mm	1% $CaCl_2$ 18 h (ratio 1:15 *w*/*v*)	5% HCl 1 h (ratio 1:15 *w*/*v*)	3% (*w*/*v*) 1 h (ratio 1:15 *w*/*v*)	Ethanol (ratio 1:1 *v*/*v*)	50 °C	18.3–23.7%, -, 0.91–1.33	[29]
Bifurcaria bifurcate; Fucus spiralis	0.5 mm		0.1 M HCl 3 h 60 °C pH 2.0 (ratio 1:20 *w*/*v*)	3% (*w*/*v*) pH 11, at 60 °C for 2 h	96% Ice-cold ethanol (ratio 1:3)	Freeze drying	24–25%, 220, -	[53]
Cystoseira barbata	0.5 mm	70% ethanol, 24 h	0.1 M HCl	3% (*w*/*w*), 60 °C 2 h	Absolute Ethanol	50 °C	19%, 126.6, -	[20]
Durvillea sp.—D. potatorum	Milled	Formaldehyde	HCl pH 1.8	pH 7	10 °C 70% and 100% isopropanol	Freeze drying	-, 44, -	[54]
Ecklonia radiata	0.25–1.4 mm	Ethanol, 3 h, (ratio 1:10 *w*/*v*)	HCl pH 1–6.5, 25–55 °C, 1–120 min	0.2 M, 45 °C, 2 h (ratio 1:20 *w*/*v*)	Ethanol (ratio 2:1)	60 °C	32.2–45.4%, 373–986, -	[33]
Lessonia flavicans; Desmarestia ligulata; Desmarestia distans		Petroleum ether, ethanol, formaldehyde		3%, 50 °C, 4 h (ratio 1:20 *w*/*v*)	96% ethanol	60 °C	11.0%, -, 1.03; 27.2%, -, 0.77; 15.2%, -, 0.58	[28]
Laminar digitata	5 mm^2–5 cm^2	2% *w*/*w* formaldehyde, 4 months	0.5 M H_2SO_4 overnight 4 °C	4% (*w*/*w*) at 20 °C	H_2SO_4 pH 2	30 °C	33–39%, 105, -	[21]
Laminaria digitata	<1 mm; 1–5 mm	2% formaldehyde overnight 60 °C	0.2 M HCl 24 h	2% at 25, 40, 60 °C	Absolute ethanol	~20 °C	-, 114, -	[17]
Laminiaria digitata	Pulverized	2% (*w*/*v*) formaldehyde, overnight	0.5 M HCl at 40 °C 1 h	Synthetic seawater with CO_2, adjust pH to 11 by NaOH/KOH or ammonia	95% Ethanol, 2 h, (ratio 1:3)	Frozen at −32 °C 12 h, freeze drying 8 h	31.1–40.5%, 85–274, -	[43]
Macrocystis sp.	1 mm		0.05–0.2 N HCl in distilled water or seawater, (ratio 1:10 *w*/*v*)	1,2,3,4% 20 °C 2 h or 60 °C 8 h (ratio 1:30)	$CaCl_2$ 2% (ratio 1:20 *w*/*v*)	Freeze drying	upto 21.5%, -, -	[37]
Nizimuddinia zanardini	Powdered	2% (*v*/*v*) formaldehyde, 24 h (ratio 1:32 *w*/*v*)	0.2 M HCl, 60 °C 3 h (ratio 1:32 *w*/*v*)	3% (*w*/*w*) 60 °C 2.5 h (ratio 1:32 *w*/*v*)	3 volume of 96%	Freeze Drying	27%, 103–119, 1.22	[24]
Non-specified	Milled	-	-	10% Choline hydroxide/NaCl eutectic solvent (1:0.5), 100–130 °C 1–5 h (ionic liquid)	Absolute ethanol		-,-,-	[46]
Non-specified		Cold fresh water	HCl	2–4% 20–40 min	HCl pH < 2.25, 26 °C, 30 min	Air dry, 37.7 °C	-,-,-	[30]
Non-specified		10% formaldehyde		1–1.2% (*w*/*w*), 1:10–15 ratio	HCl	40–55 °C	12.1%, -, -	[55]
Sargassum cristaefolium	0.25 mm	Ethanol, chloroform, water (4:2:1) overnight	HCl (pH 1–5), 25–45 °C, 30–90 min	2.5% (1:20 ratio), 70 °C 2 h	96% ethanol	Vacuum dryer 45 °C, 24 h	29.9%, 194.1, -	[27]

Table 2. *Cont.*

Seaweed Species	Size	Soaking	Acid/Alkali	Extraction [a]	Precipitation [b]	Drying	Yield (*w*/*w*), mw (kDa), M/G [c]	Ref.
Sargassum cristaefolium; Sargassum feldmannii; Sargassum ilicifolium; Sargassum polycystum		2% formaldehyde for 24 h	0.2 N HCl 24 h	2%, 3 h	Isopropanol	60 °C	0.6–1.8%, -, -	[23]
Sargassum errimum; Dictyota dichotoma; Spathoglossum asperum; Iyengaria. stellata	Milled	4% $CaCl_2$, 2 h, 50% formaldehyde 1 h	5% HCl 20 min		80% isopropanol	40 °C overnight	-,-,-	[56]
Sargassum fluitans	Ground		0.2 N HCl, 3 repeats	HCl (pH 6.5–7.5), 20 °C overnight & NaCl	Ethanol (ratio 1:1)	30–40 °C	16.3–23.4%, -, 0.5–0.6	[44]
Sargassum fluitans	Ground	5% formaldehyde	0.2 N HCl, 3 repeats	4% NaOH, 20 °C overnight (ratio 1:100)	Ethanol (ratio 1:1)	30–40 °C	18.9–21.1%, -, 0.5–0.6	[44]
Sargassum fluitans	Ground	5% formaldehyde	0.2 N HCl, 3 repeats	2%, 80 °C, 2 h		Freeze drying	18.5–24.5%, -, 0.5–0.6	[44]
Sargassum latifolium	1–5 mm		0.54% $CaCl_2$ 30 min, 0.5% NaCl 1 h 100 °C (ratio 1:15 *w*/*v*)	5% for 30 min at 100 °C (1:5 *w*/*v*)	80% Ethanol	50 °C	17.54%, -, -	[19]
Sargassum latifolium	Blended	70% *v*/*v* acetone 4 h	1 to 3% *w*/*v* citric acid, 25–45 °C, 1–3 h	2% (*w*/*v*), 40 °C for 3 h	Ethanol (ratio 2:1)	60 °C	21.10–31.78%, 142–194, 0.34–1.05	[25]
Sargassum muticum		Tap water, then frozen at −18 °C			Citric acid, pH 3	40 °C, 48 h	-, 80–112, 0.33–0.64	[57]
Sargassum muticum		0.2 % formaldehyde 24 h 16 °C (ratio 1:10 *w*/*v*)	0.2 M HCl (ratio 1:10 *w*/*v*)	0.5–5%, 1–3.5 h, 50–100 °C	50–100% ethanol	65 °C	13.6%, -, 1.08	[36]
Sargassum natans	0.5 mm	2% (*w*/*v*) formaldehyde overnight	0.1 M and 0.5 M, H_2SO_4	5–10% (*w*/*v*) 25–75 °C 0.5–6 h (ratio 1:5–15, *w*/*v*)	0.5 M H_2SO_4 pH 2, Na_2CO_3 5%, ethanol (ratio 1:15)	−17 °C 24 h & freeze drying 48 h	7–19%, -, 0.51	[18]
Sargassum polycystum	Powdered	1% (*w*/*w*) $CaCl_2$ 2 h, ratio 1:8	2% HCl 30 min	4% 90 °C (ratio 1:8 *w*/*v*)	$CaCl_2$ & Ethanol, then HCl pH 2–3	70–75 °C 8 h		[58]
Sargassum sp.	-		NaOH for 2 h	2 h 100 °C	HCl pH 2.5–3; then NaOH, & isopropanol			[35]
Sargassum sp.	Powdered	2% $CaCl_2$, 2 h 49% phenol 2 h		3% 60–80 °C	$CaCl_2$ 1–10%	Air Dry	35%, -, -	[59]
Sargassum turbinarioides		2% formaldehyde 90 °C 24 h, (ratio 1:16 *w*/*v*)	0.2 M HCl for 24 h, ratio (1:16 *w*/*v*)	2%, 3 h 100 °C	95% ethanol (ratio 1:3)	65 °C	10%, 387.9, -	[60]
Sargassum wightii; Padina tetrastromatica	Milled	4% $NaPO_2H_2$. H_2O, 24 h	0.4 M HCl, 20 °C, 2 h	4% (*w*/*w*)		70 °C		[26]

a: The chemical used the extraction step in this column is Na_2CO_3 unless specified. b: The ratio in this column refers to sodium alginate solution: ethanol (*v*/*v*), unless specified. c: Data presented in this column refer to yield (*w*/*w*), mw (kDa), M/G respectively. E.g., 27%, 103–119, 1.22 refer to 27% yield, molecular weight 103–109 kDa, M/G ratio of 1.22. "-": no data.

3. Green Alginate Extraction Process

With the increasing focus on sustainable development, many greener technologies have been developed and applied in the biopolymer extraction field [61]. Several of these technologies, such as ultrasound-assisted extraction [42], microwave-assisted extraction [62], enzyme-assisted extraction [63] and extrusion-assisted extraction [64] are used in alginate extraction from brown seaweeds with the aim to increase the alginate extraction efficiency, reduce energy consumption and minimize waste stream generation.

3.1. Ultrasound-Assisted Extraction (UAE)

Ultrasound-assisted extraction is considered to be a novel extraction technique with low solvent requirements, environmental friendly, easy handling and fast extraction rate [65,66]. It can be used in combination with other non-conventional extraction techniques such as microwave-assisted extraction [67,68]. A UAE workflow schematic diagram is shown in Figure 3a. It consists of a sonicator probe, extraction vessel and ultra-sonicator generator. The efficiency of the UAE method is based on various factors such as the temperature, extraction time, ultrasound power, solid to solvent ratio and the biomass characteristic. Youssouf et al., 2017 investigated alginate extraction from *Sargassum binderi* and *Turbinaria ornate* with the addition of ultrasound treatment at various different pH, solid loading ratio and extraction time [42]. Under the optimum condition (pH 12 using Na_2CO_3, loading ratio of 1:100, 40 min with ultrasound at 25 kHz, 150 W), the alginate extraction yield reached 54.06%. However, the experimental data showed that extraction process is very sensitive to pH, as discussed before. The ultrasound generates physical forces such as shear, acoustic streaming, microjets, leading to disperse of the cell wall, reduction in particle size and good contact between solvent and target compounds [69].

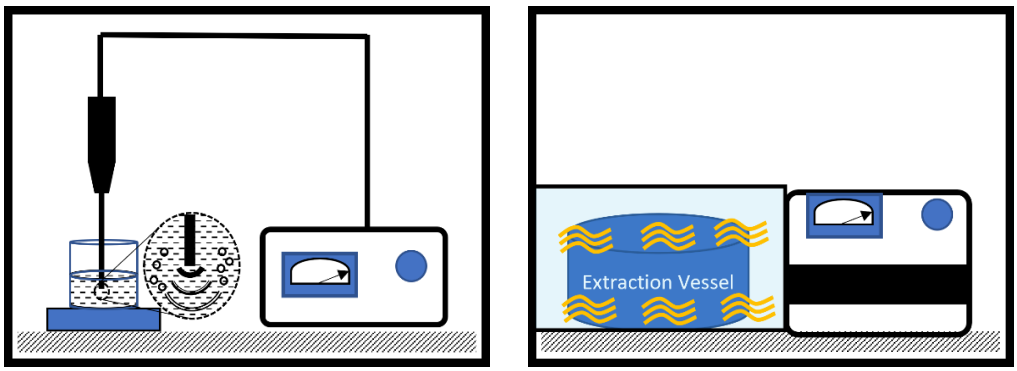

Figure 3. Schematic diagram of green alginate extraction processes (**a**) Ultrasound-Assisted Extraction (UAE); (**b**) Microwave-Assisted Extraction (MAE).

3.2. Microwave-Assisted Extraction (MAE)

MAE method has been widely used to extract active compounds in natural materials. It is considered as one of the most efficient extraction techniques compared to conventional methods. [70,71]. Figure 3b shows a schematic diagram of the MAE process for alginate extraction. During the MAE process, microwave radiation is used to increase the speed of extraction by heating the solvent efficiently and quickly. It is suggested that the heat generated by microwaves evaporates the water within the seaweed cells resulting in increased pressure on the cell wall which causes the cell wall to rupture effectively and subsequently release the intercellular compounds into the extraction solvent [72,73]. Yuan and MacQuarrie 2015 introduced the microwave heating method instead of conventional heating in the extraction of alginate [62]. Microwave irradiation was used in three stages, initial drying of fresh seaweed (*Ascophyllum nodosum*, 80–94 °C for 24 min with vacuum), pre-treatment stage with HCl (90 °C for 15 min) and extraction stage with Na_2CO_3 (100 °C for 10 min). When alginate was extracted together with the recovery of fucoidan, a yield

of 18.24% with an average molecular weight of 75 kDa was obtained. The skipping of the fucoidan step increased the extraction yield to 23.13% with a significant increase in the molecular weight of the alginate product (195 kDa). Recently, Torabi et al., 2022 explored the impact of temperature, extraction time, microwave power and seaweed loading ratio on the extraction yield in MAE method microwave-assisted extraction of *Nizimuddinia zanardini* [74]. The best extraction yield of 31.39% was obtained at a pre-treatment temperature of 67 °C, microwave power of 400 W, and a biomass loading ratio of ~1:30 (*w/v*) for 19 min. In this study, microwave heating was only used in the pre-treatment stage with HCl at pH 1.

3.3. Enzyme-Assisted Extraction (EAE)

Extraction of polysaccharides via EAE has gained growing attention; it is an environmentally friendly, non-toxic and rapid process. Enzymes specifically react with a particular substrate or a group of substrates while keeping the target products un-touched in the biomass matrix. Cellulases and proteases (e.g., Alcalase) are the most commonly used enzymes for the extraction of alginate (as well as other polysaccharides) from brown seaweed, which target celluloses and proteins, respectively [63,75]. Borazjani et al., 2017 performed alginate extraction from *Sargassum angustifolium* with the assistance of enzymes [63]. It concluded that the enzymatic treatment significantly reduced protein and polyphenol contents in the alginate product in comparison with that obtained from only dilute acid pre-treatment (0.1 M HCl, pH 2, 65 °C, 3 h, 3 times). However, the molecular weight of the alginate derived from enzyme-assisted process was reduced from 557 kDa to 356 kDa. Recently, Okolie et al., 2020 compared UAE, MAE and EAE with a conventional alginate extraction process using *Ascophyllum nodosum*. Enzyme-assisted extraction showed the highest yield, but there was no significant difference in terms of M/G ratio of the products [76]. Contrary to Borazjani et al., 2017 [63] the extract derived from the EAE process displayed a higher molecular weight (170 kDa) than that of conventional extraction process (103 kDa, 0.01 M HCl, 70 °C, 3 h, 3 times). The MAE resulted in the lowest molecular weight of 65 kDa in this case. Overall, considering the high cost of enzymes, the benefits of EAE are not promising for alginate production, although the EAE process could be further optimized to achieve higher efficiency.

3.4. Extrusion-Assisted Extraction (ExAE)

Extrusion is a thermo-mechanical process, which is traditionally used to produce food with high starch content. The production of calcium alginate bead [77] and alginate based 3D printing ink [78] using extrusion process has been widely investigated. Recently, Sugiono et al., applied extrusion technology in the extraction of alginate from brown seaweed *Sargassum cristaefolium* [64,79]. In comparison to a conventional alginate extraction protocol, an extruder was introduced at the Na_2CO_3 extraction stage to replace the beaker/reactor tank for hosting the reaction. The operation parameters, such as temperature, seaweed loading ratio, extruder feeding rate and pH were investigated. The best extraction yield observed was 45.54%, while molecular weight up to 217.94 kDa could be achieved. Interestingly, the M/G ratio of the alginate product was only 0.29, indicating a very high guluronate content in the polysaccharide [64]. As the parallel conventional extraction method was not carried out, it is not known whether low M/G ratio is due to the seaweed species or related to the specific ExAE method.

3.5. Other Possible Methods for Alginate Extraction

Besides the processes discussed in the above sections, other methods, such as pressurized liquid extraction (PLE) have been recently investigated for the extraction of valuable biochemical compounds from various seaweed species, such as antioxidant [80], antiviral compounds [81], carrageenan [82] and fucoidan [83]. The PLE method uses elevated temperature (50–200 °C) and pressure (50–150 atm) to extract compounds from solid or semi-solid samples within a short frame of time (5–15 min), which is considered as an

environmentally friendly "green" technology [81]. Co-production of alginate using the leftover solid residues in the PLE method could possibly reduce waste generation and improve the economic feasibility of the process.

3.6. Comparison of Green Alginate Extraction Processes with Conventional Processes

The comparison of greener alginate extraction processes with conventional alginate extraction processes was carried out, as shown in Table 3. The pros and cons of individual steps in the conventional processes were also summarized in Table 3. Overall, as conventional processes have been investigated for over a century, the technology is relatively mature although innovations are required to further improve the process efficiency. Green extraction processes provide promising environmental friendly options, but their industrial applications have not been demonstrated in commercial scale yet.

Table 3. Comparison of green alginate extraction processes with conventional extraction processes.

Processes	Pros	Cons
Conversional process		
with fine milling	Increase surface area, increase reaction/extraction rate, reduce reaction time	Extra energy cost, may affect molecular weight of alginate
with formaldehyde soaking	Soften cell well, remove phenolic compounds, remove pigment	Toxic chemical, need treatment before deposal, long process time
Sodium alginate extraction route	Fewer steps, high yield, good rheological properties	High organic solvent usage, high water footprint
Alginic acid extraction route	Lower solvent usage, low cost	Longer steps, low yield,
Calcium alginate extraction route	Lower solvent usage, low cost	Longer steps, poor mechanical properties
Green process		
Ultrasound-Assisted Extraction (UAE)	Low solvent requirement, environmental friendly, easy handling, fast extraction rate	Additional equipment cost, further investigate is required to demonstrate the benefits
Microwave-Assisted Extraction (MAE)	Fast heating, fast extraction, environmental friendly	Concern of scale up
Enzyme-Assisted Extraction (EAE)	Environmentally friendly, non-toxic and rapid process	Enzyme is expensive, enzyme is sensitive to extraction conditions
Extrusion-Assisted Extraction (ExAE)	Good mixing, high solid ratio, low solvent requirement	Concern of scale up, lack of investigation

4. Alginate Industrial Application

Alginate has several unique properties, such as high viscosity in aqueous solutions, biocompatibility, biodegradable, anti-microbial and therefore has been utilized in many fields such as pharmaceutical, food, cosmetic and textile industrials [84,85]. A recent study conducted by Zhao et al., 2020 showed increase in research trend on alginate and other polysaccharides over the last ten years due to growing awareness of using sustainable materials to replace fossil resource based products [86]. Alginates are widely used in medical applications as scaffolds, cell encapsulation, wound healing and for controlled drug release [87]. The presence of bioactive compounds such as amino acids, flavonoids, phenols, alkaloids and the structure of alginate itself give the products antimicrobial, antioxidant, anticancer and immunostimulatory properties. Alginate can be used as a drug carrier, due to its fabrication into a wide range of forms such as nanoparticles, films, foams or fibers [88]. An emerging application of alginate in the medical field is to develop a system to achieve multi-drug therapy within a single alginate capsule, which could lead to potential enhancement of therapeutic efficiency compared to single drug use [89]. In terms of food application, alginate can be used as an edible coating to maintain the flavor, increase the

water barrier, prevent microbe contamination, reduce fat oxidation and therefore extend shelf life of foods [69]. Sodium alginate is considered as a glycemic controller to regulate food in-take and appetite [84]. Food and Agriculture Organization and World Health Organization certified alginate as one of the safest food additives [84,90]. Alginate is also used for making jellies or desserts due to its colloidal properties, which can form insoluble polymer or a hydrogel by cross linking with calcium ions in $CaCl_2$ solution [71]. The commercial products of alginate in pharmaceutical and food applications have been recently reviewed in several papers [71,91–93]. Besides pharmaceutical and food application, alginate has also been explored for its application in wider fields, some examples have been summarized in Figure 4, such as wastewater treatment, rubber production and biofertilizer.

Figure 4. Schematic diagram of industrial application of alginate [4,94–106].

5. Sustainability of Alginate Extractions

The growing popularity of alginate usage in various industries has accelerated the production of alginate worldwide. The sustainability of seaweed cultivation and its potential to produce bioethanol, biomethane, biofertilizer and other value-added products have been intensively investigated using life cycle assessment method [107–109]. The benefits of a seaweed based biorefining process on the carbon dioxide remediation, fossil fuel replacement, marine eutrophication deduction and acidification reduction have been widely recognized. However, to secure future sustainability in the seaweed industry, it is required to improve seaweed disease control and to increase conservation of genetic diversity in wild seaweed to screen out or to genetically design promising new varieties [110,111].

Another challenge of using seaweed as a feedstock for bioprocessing is the relatively low annual output of the seaweed. The annual seaweed production of 34.5 million tonnes is low in comparison to other potential biomass resources, such as wheat straw (529 million tonnes) [112], sugarcane bagasse (279 million tonnes) [113] and citric waste (110–120 million tonnes) [114], as shown in (Figure 5). It suggests that improving aquaculture of seaweed should be supported.

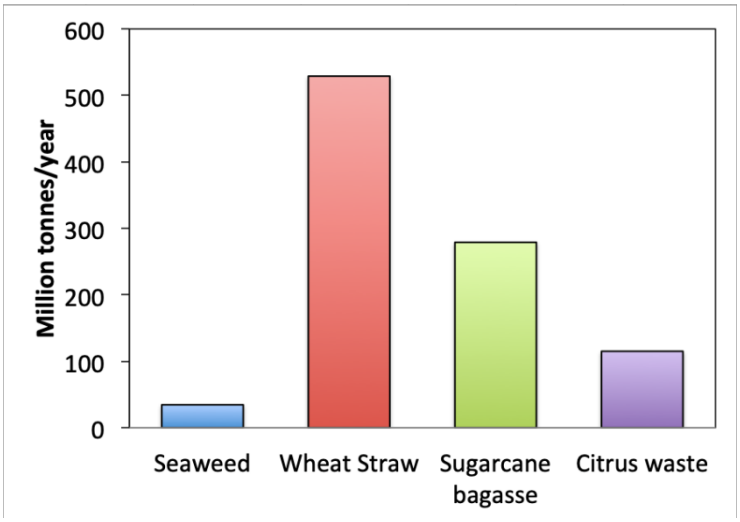

Figure 5. A direct comparison of alginate annual production with other common biomass resources.

Although a direct assessment of alginate extraction on the environmental impact has not been reported yet, it is rational to claim similar environmental benefits related to the seaweed cultivation area. However, current industrial alginate extraction processes contain several drawbacks, such as utilizing significant amount of chemicals and water as well as it been an energy intensive process. These concerns could potentially limit the sustainable production of alginate in the future. As discussed in Section 3, conventional alginate extraction processes require large amounts of acid and alkali (Table 2), which remain in effluent at the end of the process. Furthermore, toxic chemicals, such as formaldehyde are commonly used in the soaking process to facilitate the subsequent pre-treatment process. The excess use of chemicals increases the environmental burden of the alginate production processes. Sodium alginate can dissolve in water but forms a viscous solution. With the increase of alginate concentration, the viscosity of the solution increased significantly [115]. Therefore, a significant amount of water is required to dilute the sodium alginate solution, e.g., to reduce the alginate concentration to no higher than 2% (*w*/*w*) to enable it: (i) movable for mixing in the alkali extraction step; (ii) filterable in the separation step and (iii) transportable between different vessels. As shown in Table 2, in a typical alginate extraction process, water is added in the soaking, acid/alkali pre-treatment and extraction process with a solid loading ratio of at least 1:8 (*w*/*v*), commonly in the range of 1:10–20 (dry weight biomass/filtration residue: solution). A techno-economic assessment of a typical alginate extraction process was carried out in the authors' group, which showed that the around 47.0 L of water is used to produce 1 kg of dry weight alginate in a conventional alginate extraction process (unpublished data). This value is significantly higher than other industrial products, such as bioethanol 5–11 L/L [116] and beer 5–6 L/L in 2006 [117]. The higher water footprint could potentially hinder the large-scale alginate production. The usage of organic solvents is another concern for sustainable alginate extraction. Organic solvent, mainly ethanol is used to precipitate sodium alginate from its aqueous solution. The volumetric ratio of ethanol to sodium alginate solution is normally in the range of 1:1 to 1:3 (Table 2). As the sodium alginate solution is low in concentration and high in volume, the amount of ethanol required is high as well. Although ethanol can be recycled

via distillation, it is an energy intensive process and is not economic feasible for small-scale alginate production. Therefore, integrating an alginate extraction process with an existing bioethanol production plant should be considered.

6. Conclusions and Future Efforts

Alginates are promising biodegradable and renewable biopolymers, which have been widely applied in pharmaceutical, food, textile and cosmetic industries. Since the first approach for alginate extraction was published, the process has been adapted to fit alginate extraction from various seaweed species. Although the alginate yield and quality is highly dependent on the seaweed species, the optimisation of extraction parameters, such as soaking medium, pre-treatment chemical and protocol, extraction temperature, time and pH, and precipitation reagent, could improve the extraction efficiency significantly. The recently developed greener technologies, such as ultrasound-assisted extraction, microwave-assisted extraction, enzyme-assisted extraction, and extrusion-assisted extraction have been explored to aid alginate extraction, leading to enhanced extraction yield and accumulation of production with desired properties. For the sustainable production of alginate, the alginate extraction process should be optimised to reduce energy input, decrease water footprint, minimize carbon footprint and integrate alginate extraction with the production of other value-added products.

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