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Characteristics of Liquid Product of Alkaline Treated *Sargassum polycystum* C.A. Agardh. from Lange Beach, Aceh

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Abstract

Sargassum is known to have potential ingredients, such as essential nutrients and minerals, which are important for agroindustry and the environment. This study aims to determine the proximate properties of Sargassum polycystum C.A. Agardh from Lange beach and the optimum extraction period arising the liquid product. The dried seaweed was extracted using 0.1% KOH at a constant temperature of 80°C for 120–600 mins. Furthermore, nutrients content, pH, electrical conductivity (EC) and total dissolved solids (TDS) of the afforded filtrate were quantified. As a result, the moisture, ash, fibre, carbohydrate, protein, and fat content were 7.16, 13.057, 20.33, 51.77, 6.12, and 1.57%, respectively. The value of pH, EC, and TDS ranged around 4.69–7.27, 0.013–0.078 dS.m^{-1}*, and 6.24–39.75 mg.L*$^{-1}$*, respectively. The level of N, P, K, and organic C, were estimated around 0.30–0.81, 0.16–0.28, 0.15–0.20, and 0.32–0.80%, respectively. Meanwhile, the content of Na, Mg, Ca, Fe, Mn, and Zn were observed from 112.04–212.61, 24.60–33.57, 146.59–280.92, 416.22–630.69, 18.25–27.73, and 19.86–26.53 ppm, respectively. In conclusion, extraction for 120 mins seems to be preferable, as the observed parameters were above the minimum range.*

Keywords: *proximate, brown seaweed, liquid fertilizer, micro- and macronutrients*

Introduction

In 2018, Food and Agriculture Organisation (FAO) of the United Nations (2018b) acknowledged Indonesia as the second major worldwide supplier of seaweeds contributing as 38% in the global trading development (FAO, 2018a). The national production increased dramatically from less than 4 million to 10.456 million tonnes in 2010 to 2017, respectively, which was valued as IDR 21 trillion (Pusdatin, 2018). The farming activity produced such enormous values has attempted only at a half-scale of all potential resources, approximately 384.7 thousand Ha used from 769.5 thousand Ha (KKP, 2013). Therefore, an opportunity to maximise the economic commodity could be taken into account.

One of the unutilised farm space allowing a brown seaweed, *Sargassum*, is Lange beach situated in Lam Lhom, a rural area in Aceh Besar. It has been reported that raw material for manufacturing brown algae-based hydrocolloid, alginate, is in globally high demand, as much as 560 thousand tonnes per year, and worth US$ 40 million (Nayar and Bott, 2014). Both the local and national communities could benefit from this resource by developing a practical framework to cultivate the marine plant and innovate profitable management systems of the by-products. Due to no scientific study of the species from the aforementioned location, a preliminary work including proximate profile seems to be crucial to underpin further action needed to decide the sustainable economic implementation.

Although domestic alginate manufacturer has been established since 1993, the volume of production has less contribution to the national income compared to the other phycocolloid industries, carrageenan and agar. Moreover, while the national order of alginate tends to increase every year, the commercial rate of the product is relatively more expensive than the two marine saccharides. In 2015, it was 3 times higher than agar-agar, and 4 to 6 times higher than carrageenan (Salim and Ernawati, 2015). In addition, some challenging factors such as lack of infrastructure to access the habitat of *Sargassum*, unadvanced cultivation technology apply, and less innovation in by-products management system are likely to be considered as the main causes of the circumstance

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(Salim and Ernawati, 2015; Muslimin and Sari, 2017).

Darmawan *et al*. (2006) described two opposite effects of the application of alkaline treated sargassum (ATS) using KOH 0.1% as the primary solvent in the alginate production as following as either improving the quality of sodium alginate or generating liquid disposal. However, its waste can be applied in a further process converting it to sargassum liquid fertilizer (SLF) (Spurr, 2014 and Basmal *et al*., 2015). Scientifically, Sargassum thallus has been reported as the source of macro- and micronutrients, humic acid, tannin, iodine, growth regulators (auxins, gibberellin, cytokinin-kinetin, cytokinin-zeatin), phenol and vitamin (Basmal *et al*., 2017). Furthermore, the absence of lignin layer in the plant affects to let the decomposition of essential substances for both crops and soil run smoother (Spurr, 2014).

Some studies have revealed enormous potential advantages of *Sargassum* ingredient to agroindustry and the environment. High level of essential nutrients and minerals has been associated to the effects of *S. polycystum* (Bharath *et al*., 2018) and *S. vulgare* extract (El-Din, 2015) promoting the growth and productivity of leguminous plants; the improvement of photosynthesis pigments, protein, sugar, and ascorbic acid production as well as inhibition of nitrate reductase enzyme demonstrated by *S. illisifolium* extract (Pise and Sabale, 2010a) and *S. wightii* extract (Vijayanand *et al*., 2014; Divya *et al*., 2015; Divya and Reddi, 2017), stimulation of seed germination provided by *S. pallidum* (Turner) C. Agardh (Anisimov and Chaikina, 2014), *S. vulgare* (Salma *et al*., 2014) and *S. myricocystum* (Sujatha *et al*., 2015). Salinity, another important component, commonly expressed in pH, EC, or TDS, has also been closely related to the positive properties of the macroalgae extract. For instance, aqueous extracts of *S. wightii* and *S. johnstonii* with pH and EC as 7 & 7.5 and 6.40 & 6.20 dS/m, respectively, showed beneficial impacts on both chlorophyll and primary metabolites production and soil nutrition and moisture maintenance (Takoliya *et al*., 2019 and Patel *et al*., 2019). In addition, advantageous effects toward plants such as increasing the rate of growth and development and improving the volume and the mass of crops were exhibited by two species with different degree of electric conductivity: *S. crassifolium* (Sutharsan *et al*., 2014) and *S. muticum* (Silva *et al*., 2019) with pH and EC value as 9 and 6.6–7.22, and 4 and 0.1–0.2 dS.m^{-1}, respectively.

With regards to ATS by-products study, Basmal *et al*. (2010) reported that heating *Sargassum* sp. at 80^{o}C for 360 mins afforded the highest level of micronutrients and salinity parameters compared to three other shorter heating periods: 0, 120, and 240 mins. However, the research did not show the optimum extraction time that allows any observers to record the peak period characterised by a pointed top level of the achieved components. Therefore, along with proximate study, this work was conducted by adding two longer extraction time, 460 and 600 mins, to measure the essential components needed to classify the quality of the liquid product of Lange brown algae.

Materials and Methods

Preparation and proximate analysis

S. polycystum was taken from Lange beach in Lam Lhom (Aceh Besar) at 5°31'02.6"N and 95°12'04.5"E. The fresh seaweed was rinsed with flowing water three times to separate undesired particles from the thallus. Afterwards, the sample was sun-dried for three days, followed by oven-dried at 60^{o}C overnight. The dried *Sargassum* was powdered using a blender and then subjected to proximate analysis. The measurement of moisture, ash, fibre, carbohydrate, protein, and lipid content was carried out following AOAC methods (2015). All data were expressed in % (w/w).

Extraction

The alkaline-based method described by Basmal *et al*. (2017) was adapted with a slight modification by putting a longer different time of extraction in place. The study employed six types of samples, including one control and five heat-treated samples. All samples were prepared by adding fine powdered *Sargassum* into KOH 0.1% with a ratio of 1:10 (w:v). Filtrate 1 as the control was obtained by filtering the mixture which was soaked for 60 mins at the room temperature. Meanwhile, five other samples – namely, filtrate 2, 4, 6, 8, and 10 – were the liquid product of the filtration of the mixture heated at 80^{o}C for 120, 240, 360, 480, and 600 mins, respectively. Analysis of salinity properties and nutrients content were applied to all samples.

Measurement of pH, EC, and TDS

The pH value was determined using a digital pH meter while the EC value was estimated with an electrometer. The TDS measurement was observed through the gravimetric method.

Analysis of nutrient content

The total nitrogen content was measured using the procedure SNI 4146:2013. The sample was digested with boiling concentrated H_2SO_4 to obtain a green product. The mixture was then distilled to separate a liquid placed into a conical flask added with H_3BO_3 4% and indicator MR and BCG. The distillate was subjected to a titration using HCl 0.1 N to reach endpoint (green-yellow). The level of N was calculated using the acid volume needed to neutralise the sample.

The analysis of organic C content employed the protocol of BPT (2005). The filtrate was added into concentrated H_2SO_4 and $K_2Cr_2O_7$ 1 N and then stored for 30 mins. Into the mixture, concentrated H_3PO_4 was added and homogenised. The two dropwise of indicator DPA was added into the solution and titrated with $FeSO_4$ to reach the endpoint (gree-blue). The concentration of C was estimated using the solution volume needed to neutralise the sample.

The concentration of P and K was calculated using the protocol of BPT (2005). Standard solution of P_2O_5 was prepared in 0, 2.5, 5, 7.5, 10, 12.5, and 15 ppm while the standard solution of K_2O was made in 0.5, 10, 15, and 20 ppm and then observed in spectrophotometer UV-vis at 650 nm wavelength. Into the sample, concentrated HNO_3 and $HClO_4$ was added and heated. After filtration, the resulted filtrate was diluted with aquades, added by HNO_3 2 N, and ammonium heptamolibdat vanadate solution and homogenised. The mixture was observed under spectrophotometer UV-vis at 650 nm wavelength and then compared with the absorbance data of P and K standard to estimate the concentration.

The level of Na, Mg, Ca, Fe, Mn, and Zn, were determined using the procedure of AOAC (2015). Each standard solution of the minerals was prepared in 0, 0.1, 0.5, 1, 2, 3, and 4 ppm. The standard curve was made through atomic absorption spectroscopy (AAS) at wavelength number of 589.6 nm (Na), 285.2 nm (Mg), 422.7 nm (Ca), 248.3 nm (Fe), and 279.5 nm (Mn and Zn). Into the filtrate, concentrated HNO_3 was added and then heated exhaustedly. The sample was diluted with aquades up to 100 mL. The absorbance of the sample was measured at the proper standard wavelength depending on the type of the mineral while the concentration was calculated by comparison between the absorbance of the sample and the proper standard curve.

Results and Discussion

Proximate profile

In the solvent-based extraction, the permeability of molecules of the solvent affecting to the penetration into cell wall appears to be mainly driven by the water content of the sample since H_2O molecules could compete to the solvent. The higher permeability of the solvent tends to allow a higher concentration of substances to be extracted. Both organic and inorganic components stored in the tissue could be withdrew using intermolecular force performed by solvent molecules. However, H_2O molecule can lead to interference of substance-solvent interaction due to the presence of hydrogen bonding. Therefore, an effective drying method is an important preparation step to run a maximum rate of water evaporation. In this study, both sun and oven-drying were applied to reduce an ideal volume of water, 93%, as reported by Holdt and Kraan (2011). These techniques were developed by adapting Masduqi *et al.* (2014) that demonstrated evaporation under sunlight following oven at 60°C as the effective method of seaweed drying. As a result, the moisture content of the sample could be classified to the lower content compared to the other species *Sargassum*.

Ash content can be used as a primary manner to determine the purity degree of a sample. Ash could be considered as the remaining inorganic materials from a process of sample combustion. The ash content of Lange brown algae is likely to be categorised as lower level compared to the other species and common level range for *Sargassum* as documented, i.e. 14–44% (Holdt and Kraan, 2011). However, its value was fairly similar to the *S. oligocystum* (Manteu *et al.*, 2018). Generally, geographical characteristics of the habitat of the organism and the climate and season, which are varied strongly affect the diverse reports of the content.

Table 1 Proximate composition (%) of *S. polycystum* C.A. Agardh. in dried weight compared to references

Parameter	*S. polycystum*		*Sargassum* sp[2]	*S. oligocystum*[3]	*S. vulgare*[4]
	Lange	Pohuwato Gorontalo[1]	Lhok Bubon, Aceh Barat	Kenya coast	India
Moisture	7.16	17.69	10.54	6.49	-
Ash	13.057	24.51	52.74	13.08	19.4
Protein	6.12	3.65	2.53	5.64	13.6
Carbohydrate	51.77	53.66	23.77	71.42	61.6
Lipid	1.57	0.55	0.79	0.46	0.5
Fibre	20.33	6.52	-	9.4	-

Sources: [1]Manteu *et al.* (2018), [2]Gazali *et al.* (2018), [3]Muraguri *et al.* (2016), and [4]Kumar *et al.* (2008)

With the salinity reported as 3.15–3.25% in July (Marghany, 2014), the habitat along Lange beach could be considered as the quite less saline water compared to normal seawater with salinity as 3.5%.

The protein content of the marine plant showed a relatively higher level than the species from the literature. Since the protein levels of *Sargassum* are between 0.3 and 5.9% (Erniati *et al*., 2016), the sample in this study might be reported as higher protein algae due to its content of 6.12%. Therefore, this finding appears to empirically justify *S. polycystum* from Lange as a potential source of protein from which the nitrogen species could come up through the decomposition process. Theoretically, the protein in *Sargassum* predominantly consists of glutamic acid and aspartic acid as much as 39–41% of its wet weight (Holdt and Kraan, 2011). However, further analysis is needed to confirm the composition of the amino acids making the algae up.

The carbohydrate content of *S. polycystum* reflects the standard range of *Sargassum* reported by Holdt and Kraan (2011), around 54.3-73.8 %, but is considered to be low in Erniati et al. (2016), and extremely high in Venugopal (2011) as their standard ranges are 4–68% and 15-20%, respectively. This species is likely to be the richness of biomass needed for other applications such as raw material for alginate, fucoidan, and laminarin. Such number also indicates that the algae could potentially be nourishing plants and soil because, generally, *Sargassum* polysaccharides contain abundantly sulfurous groups.

S. polycystum of Lange beach contain relatively higher lipid level compared to the literature where the value was doubled higher than those. On the other hand, this species has a slightly lower lipid level than *S. crassifolium* growing in Gorontalo with a fat content of 1.63% (Handayani *et al*., 2004). When compared to the Venugopal (2011) report on *Sargassum* in colder subtropical regions, Lange's species is 0.43% lower than the minimum range of the documented levels (2-4%). This content is in agreement with Kumar *et al*. (2008) arguing that the tropical *Sargassum* fat content is <1% lower than in colder temperatures.

The brown seaweed from the studied site was found rich in dietary fibre formed by soluble and insoluble fibre. This highlights the value of the species as a prospective source of bioactive polysaccharides and biofertilizer. The macroalgae could moisturise and maintain soil fertility through promoting aeration rate and enriching soil structure. In addition, cellulose, mannan, and xylan are likely to be extracted in sufficient quantities used as active ingredients for plant growth. The level of *Sargassum* fibre from Lange was almost three times higher than the species of Poluwatu. However, when compared to the data from Holdt and Kraan (2011), the levels were underneath the reported range of 33-62% yet.

Analysis of pH, EC, and TDS

The liquid product of ATS can be characterised using electrolyte parameters including pH, TDS and EC. Based on the measurements, the pH of liquids 1, 2, 4, 6, 8, and 10 were 6.90, 6.73, 6.29, 4.69, 7.27, and 5.22, respectively. The fluctuated pH range might be associated with the organic C levels representing the extracted saccharides. Higher C concentrations could lead to more acidic pH because the level allows the sample to be easily oxidised (Phibunwatthanawong and Riddech, 2019). This liquid product might be classified as the good product due to relatively high content of electrolytes ions indicated from pH range of 4-8 (Regulation of Ministry of Agriculture Republic of Indonesia (RMARI) number 70/SR./140/10/2011, and Seswati *et al*., 2013). According to Sutiyoso (2003), extracts possessing higher ion content tend to modulate plant growth.

As presented in Figure 1, the highest EC value goes to filtrate 2 afforded by heating for 2 h. With a value of 0.078 dS.m^{-1}, the filtrate should be safe for all types of plants including agricultural commodities that are sensitive to a saline environment. This liquid could be applied for hydroponic plants. Generally, the EC and TDS patterns in Figure 2 are completely alike. Those show that the number of dissolved solids in this study contributed to the value of electrical conductivity positively. On the other words, the dissolved particles observed were charged minerals consisting of cations and anions. Grace (2016) stated that TDS value is closely interrelated to EC. These findings are essential to suggest the presence of minerals commonly constituted the genus. Regarding the estimation of EC and TDS, this extract might be able to prevent yellowing leaf and stunted plants due to lower value (≈0) and higher of the EC (>5 dS.m^{-1}) (Grace, 2016).

Analysis of essential nutrients

Macronutrient analysis results illustrated in Figure 3 represent necessary nutrient components needed to support their plants' growth. Compared to RMARI number 70, the contents of this product were relatively lower. Further research is needed to increase the levels of these macroelements to meet the minimum standards. On the other hand, all macronutrient levels in this study were somewhat higher than those from Sutharsan *et al*. (2014), Basmal *et al*. (2017), and Barath *et al*. (2018).

A liquid extract of S. *crassifolium* with N, P, K levels of 0.04, 0.0009, and 0.15% has been confirmed to promote both the growth rate of major organs and the volume and quality of hydroponic fruit plants (Sutharsan *et al*., 2014). With regards to the higher levels above 0.1%, the extract of this research can be categorised as a potential biofertilizer. According to the Figure 3, heating for 2 h produced an extract with the highest levels of N. A relatively shorter period of heating could prevent the denaturation of the peptide bonds which cause the lowering solubility in water. In contrast, extract 2 has little gaps toward the extracts with the highest P and K levels, specifically 0.03 and 0.01% lower. Additionally, for organic C level, extraction for 360 mins resulted in the highest levels. The C/N values for liquid 1, 2, 4, 6, 8, and 10 were 0.716, 0.642, 0.9, 2.5, 1.667, and 1.083, respectively. These ratios are still below the criteria of Permentan no. 70, which is at least 6.

Figure 4 shows the data of essential mineral contents in the extract. Briefly, the mineral content of Na, Mg, Ca, Zn, and Mn were under the range of the RMARI number 70, which are 250-5000 ppm. When compared to similar species from the Gorontalo Pohuwato coast (Manteu *et al*., 2018), *S. ilicifolium* ingredients in brown seaweed indicated by the

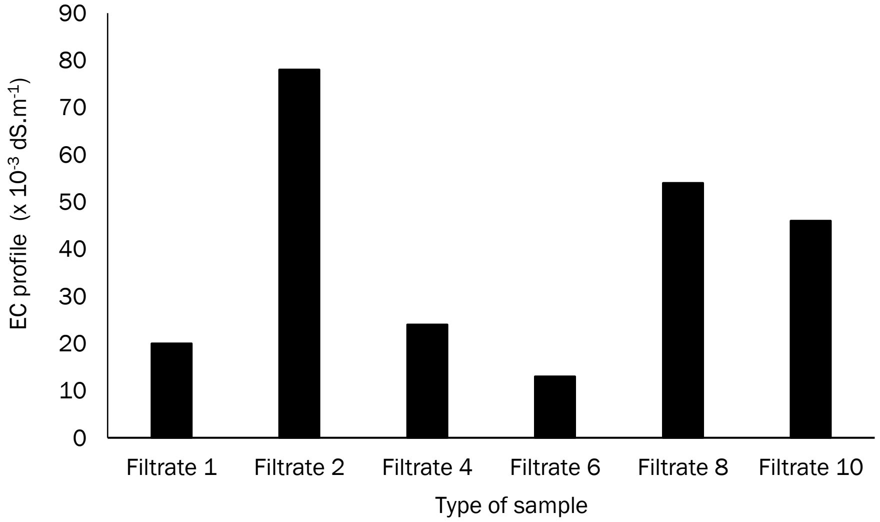

Figure 1 EC profile of six liquid products extracted from *S. polycystum* from Lange beach

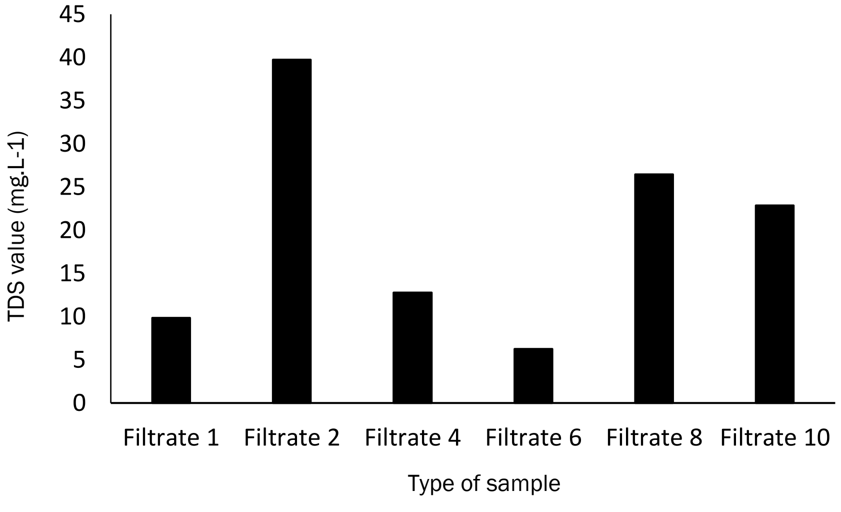

Figure 2 TDS profile of six liquid products extracted from *S. polycystum* from Lange beach

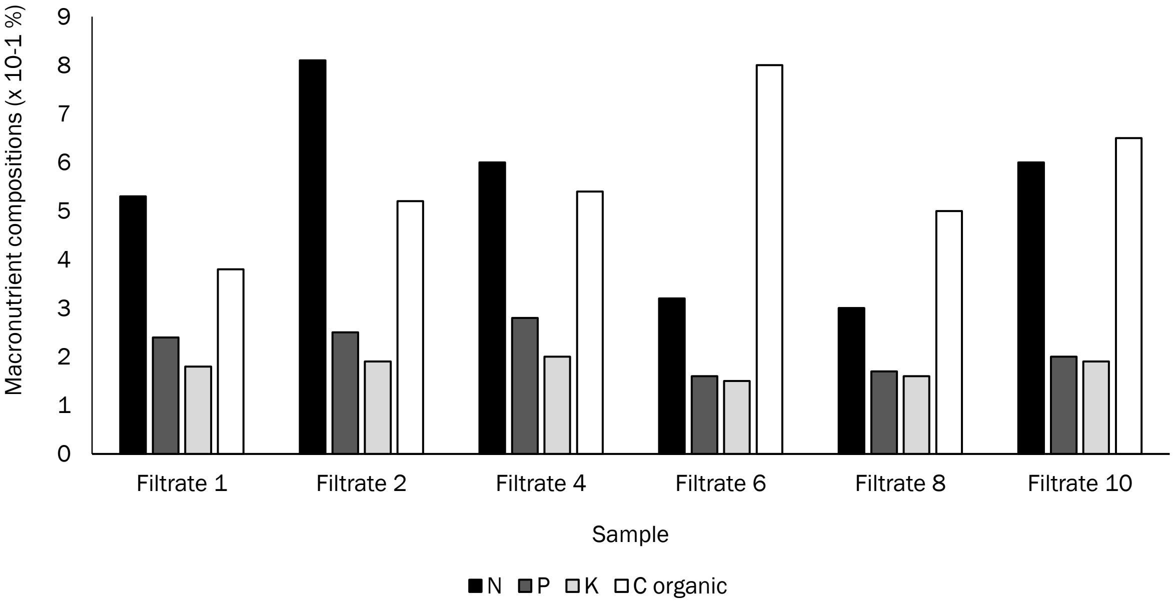

Figure 3 Macronutrient compositions of six liquid products extracted from *S. polycystum* from Lange beach

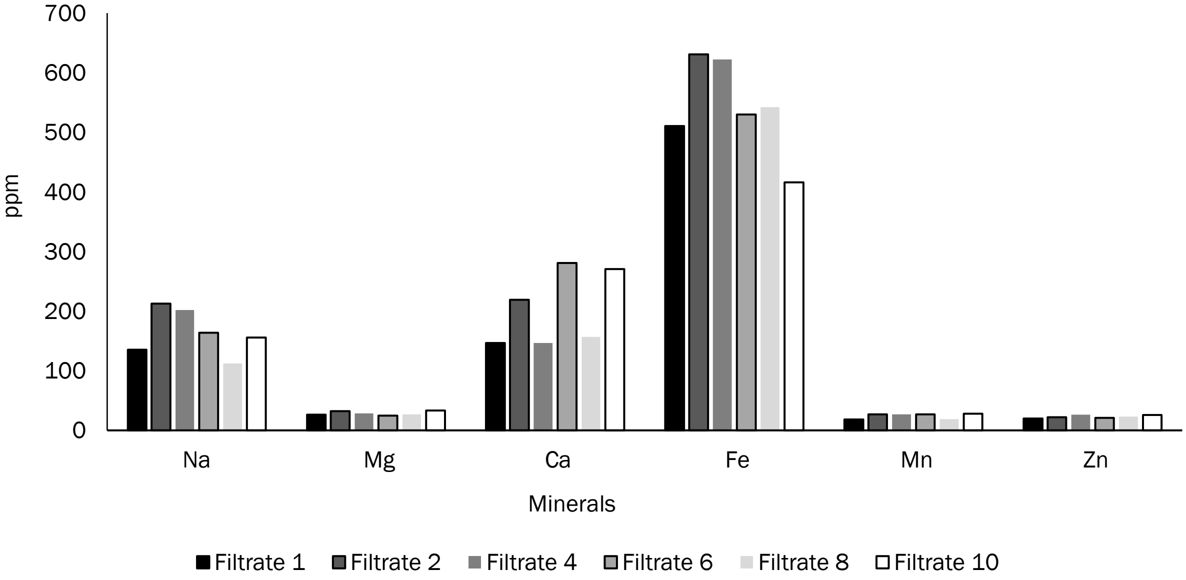

Figure 4 Micronutrient compositions of six liquid products extracted from *S. polycystum* from Lange beach

Arabian sea (Pise and Sabale, 2010b), and *S. crassifolium* Sri Pasikudah Beach (Sutharsan *et al*., 2014), the levels were higher because the sampling carried out at the peak of the dry season might affect to the quantification. Tropical *S. polycystum* is well recognised to be more productive in the summer with cooler temperatures (Noiraksar *et al*., 2017). Further research investigating the influence of location factors and sampling season is needed to clarify the hypothesis. Based on the Fe contents, all liquids possess values above the RMARI number 70 range of 90-900 ppm. Mostly, the mineral content data show that extraction for 2 h produced the highest concentrations except Ca and Zn.

This research found that heating for 2 h was the optimal time in the extraction of *S. polycystum* with 0.1% KOH at 80°C. This agrees with the report recording such period as the ideal time of commercial alginate manufacture (McHugh, 2003). A well-qualified SLF is reported to have pH and EC value no more than 7 and 20 dS.m^{-1}, respectively (Phibunwatthanawong and Riddech, 2019). The heat treatment during that time effectively extracts vital relatively higher levels of macro- and micronutrients. The longer heating time could probably dissolve more organic macromolecules into the extracts indicated by the amount of organic C levels in Figure 3 that can lead to reducing the concentration of essential substances. Consequently, the macromolecules as the nonpolar species may lower the electrical conductivity because they could disrupt ion-ion interactions in the system.

Compared to Basmal *et al*. (2017), the results of this study are extremely different. In terms of heating time, the authors confirmed that the longest heating time, 6 h, produced the best product. Based on their EC value, the liquid may not be safe for salt-tolerant plants, especially for hydroponic species. Finally, the ecosystems and harvesting time can mainly drive the diversity of the characteristics of algae (Holdt and Kraan, 2011). These factors can be taken into account to dominantly playing in the significant difference between this study and the reference.

Conclusion

Based on the measured properties, *S. polycystum* C.A. Agardh from Lange beach has been fairly confirmed as a promising raw material for organic liquid fertilizer. The content of its proximate were in line with the typical requirements for SLF production. The most favourable time for necesary extraction at 80ºC was 120 mins by which the filtrate exhibit accepted value of pH, EC and TDS as Sargassum-based biofertilizer. Moreover, the nutrients levels were above the minimum value in each parameter as well. However, more investigations, including quantitative analysis of growing regulatory hormone as well as the application of the extract toward hydroponic crops, are needed to establish comprehensive information of the macroalgae.

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