# Measurement of macroalgae total carbohydrate content found in Lendang Luar coast, Lombok, Indonesia for potential sources of bioethanol

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# Measurement of macroalgae total carbohydrate content found in Lendang Luar coast, Lombok, Indonesia for potential sources of bioethanol

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**Abstract**. Bioethanol is a renewable alternative energy through the process of fermenting sugar from carbohydrate sources by adding microorganisms such as yeast or bacteria. Macroalgae or known as seaweed is one of the producers in the marine environment which has a high carbohydrate content so that it can be used as raw material for bioethanol. Macroalgae contains specific carbohydrates including laminarin, mannitol, alginate, agar and polysaccharides. The high carbohydrate content indicates the ethanol content produced. The aim of the study was to initial screen total carbohydrates in red algae (*G. latifolium* and *G. rugosa*) and brown algae (*M. rosea*, *S. crassifolium*, *S. cristaefolium*, *S. polycystum*, *P.australis* and *T. murayana*). Carbohydrate total analysis was performed by colorimetric assay using the BioVision kit. The overall carbohydrate content found in macroalgae samples in this study was 28.23 $\mu$g $(DW)^{-1}$. The highest carbohydrate content was obtained by *G. latifolium* (37.50 $\mu$g $(DW)^{-1}$), followed by *G. rugosa* (34.27 $\mu$g $(DW)^{-1}$) and *S. cristaefolium* (33.33 $\mu$g $(DW)^{-1}$). Current results show that macroalgae exhibits sufficient amounts of carbohydrate which could potentially be further developed as source for biotehanol.

**Keywords:** bioethanol, BioVision kit, carbohydrate, seaweed

## 1. Introduction

Macroalgae (seaweed) are primary producers in the marine environments. Macroalgae is different compared to terrestrial plants based on biochemical compositions due to their various carbohydrate content from lignocellulosic biomass such as laminarin and mannitol [1]. Algal biomass is rich in carbohydrates so it has great potential to be used for the production of fuels such as biohydrogen,

biodiesel and bioethanol [2,3]. Macroalgae has several advantages when used as raw material for bioethanol because macroalgae have relatively high growth rate, high carbohydrate content, eco-friendly, not competing with food crops and cultivable land [4].

Bioethanol is derived from biological source simple sugars, cellulose and starch such as sorghum, sweet potato, wheat straw, wheat bran, corn stover and macroalgae. Macroalgae have been identified and classified into three groups based on their pigmentation, Phaeophyta (brown algae), Rhodophyta (red algae) and Chlorophyta (green algae). The macroalgae from these different groups also exhibits different types of carbohydrate content . In Chlorophyta, carbohydrate-based polymers are found such as starch and cellulose. Rhodophyta contains the most dominant polysaccharide, namely galactan which is a combination of carrageenan and agar. Phaeophyta contains a unique type of carbohydrate, namely sulfate galactans with side chains containing non-reducing sugar alcohols (mannitol) and alginates (polymers of mannuronic and guluronic acids) [5].

The total carbohydrate content of macroalgae have an important role in the bioethanol produced [6]. Carbohydrates can be measured using a variety of methods that use an acidic environment. Under acidic conditions, polysaccharides will be converted into monosaccharides by hydrolysis at a certain temperature. Saccharification and hydrolysis are essential for converting polysaccharides into monosaccharides which are used for ethanol production [7]. Bioethanol has advantages when compared to conventional fuels because it has a higher octane and heat of vaporization. The production of bioethanol is carried out through a reducing sugar fermentation process by adding microorganisms such as yeast or bacteria [8].

The development of bioethanol is very important to pay attention to the composition of the starting material by using the right method of measuring total carbohydrates and calculating accurate results. There are several methods commonly used to measure the total carbohydrate content of macroalgae, including colorimetric, chemical, enzymatic and total by difference. This study evaluates the total carbohydrate content of red algae and brown algae by colorimetric assay.

## 2. Material and Methods

### *2.1. Preparation sample of red algae and brown algae*

Red algae species (*Gelidium latifolium*, *Galaxaura rugosa* and *Mastophora rosea*) and Brown algae species *(Sargassum polycystum, Sargassum cristafeolium, Sargassum crassifolium, Turbinaria murayana* and *Padina australis)* were collected from Lendang Luar Coast of Lombok Island (8°27'47.3"S and 116°02'08.1"E). Macroalgae samples were rinsed with water and then dried for 6 days without direct sunlight. Samples were mashed using a blender with particle size 4000 $\mu$m.

### 2.2. *Morphology of red algae and brown algae*

Morphologically, algae cannot be distinguished between leaves, stems and roots. Algae have leaf-like blades with a flat and wide shape, while stipe is a part that resembles a stem and works as a wave barrier. Holdfast is a part that resembles a root and functions as an attachment to the substrate. Distribution and morphology of macroalgae depend on environmental conditions such as light quality, substrate, salinity, temperature, pH, nutrient salinity and population level. Red algae belong to the division Rhodophyta have chlorophyll a, phyco-bilins, and some carotenoids as photosynthetic pigments. Brown algae belong to the division Phaeophyta have pigments are chlorophylls a and c and carotenoids (where fucoxanthin predominates, responsible for their brownish color) [9].

### *2.3. Determination of total carbohydrate content*

The colorimetric method outlined is a well-established quantification method that has been extensively used in many fields of research for carbohydrate quantification. The assay is based upon a phenol-sulphuric acid reaction that depends on the dehydration of hydrolysed sugars to furfural derivatives during their reaction with concentrated sulphuric acid [10, 11]. Total carbohydrates in brown algae were determined using the BioVision kit. Sample and glucose standard readings were carried out using UV-Vis Multiskan GO, Thermoscientific spectrophotometer at OD 490 nm. Samples of brown algae (0.05 gr) were mixed with 200 $\mu$l of ice cold Assay Buffer and then vortex for 5 minutes. The liquid

sample was centrifuge at 12000 rpm for 5 minutes. 5 $\mu$l supernatant was adjust volume until 30 $\mu$l per well with $dH_2O$ and 150 $\mu$l $H_2SO_4$ (98%, not provided). The liquid sample was incubated at 90ºC (500 rpm) for 15 minutes, add 30 $\mu$l of developer and mix on shaker for 5 min at room temperature. Mix the contents for 1 min and Measure OD at 490 nm. The total carbohydrate content was determined with equation (1) where **B** is the amount of total carbohydrate from Standard Curve (glucose equivalents) and **V** is the sample volume added into the reaction well ($\mu$L).

**Total carbohydrate concentration in the sample = B/V x Dilution Factor = µg/µl or mg/ml (1)**

*2.4. Statistical Analysis*

Statistical analysis was conducted using GraphPad Prism version 9.2.0 with one-way analysis of variance (ANOVA) followed Tukey test with 95% of confidence level. A significant value of <0.05 was considered statistically different (GraphPad Software, Inc).

## 3. Results and Discussion

*3.1. Macroalgae species used as samples for carbohydrate total analysis*

Red algae and brown algae generally grow in the middle to lower littoral zone in deep sea. In addition to the biochemical composition of macroalgae, there are several influences of environmental factors such as light intensity, temperature, wind, salinity, pH, and nutrient concentration in the waters. Ecological parameters fluctuate with reference to the geographical location and season of sampling. The tidal period greatly influences the availability of macroalgae biomass and biochemical composition [12]. Description of macroalgae samples used in this study is shown in Table 1.

**Table 1.** Macroalgae species and sampling location

| Sample | Location | ID Voucher | Latitude | Longtitude | Division |
|---|---|---|---|---|---|
| *Gelidium latifolium* | Lendang luar | IDN_LL_09 | 8°27'47.3"S | 116°02'08.1"E | Rhodophyta |
| *Galaxaura rugosa* | Lendang luar | IDN_LL_10 | 8°27'47.3"S | 116°02'08.1"E | Rhodophyta |
| *Mastophora rosea* | Lendang luar | IDN_LL_11 | 8°27'47.3"S | 116°02'08.1"E | Rhodophyta |
| *Sargassum crassifolium* | Lendang luar | IDN_LL_01 | 8°27'47.3"S | 116°02'08.1"E | Phaeophyta |
| *Sargassum cristaefolium* | Lendang luar | IDN_LL_02 | 8°27'47.3"S | 116°02'08.1"E | Phaeophyta |
| *Sargassum polycystum* | Lendang luar | IDN_LL_03 | 8°27'47.3"S | 116°02'08.1"E | Phaeophyta |
| *Padina australis* | Lendang luar | IDN_LL_06 | 8°27'47.3"S | 116°02'08.1"E | Phaeophyta |
| *Turbinaria murayana* | Lendang luar | IDN_LL_07 | 8°27'47.3"S | 116°02'08.1"E | Phaeophyta |

*3.2. Determination of total carbohydrate content of macroalgae samples*

The determination of total carbohydrate content with macroalgae species are shown in Figure 1. The overall carbohydrate content found in macroalgae samples in this study was 28.23 $\mu$g $(DW)^{-1}$. The genus *Gelidium* is known carbohydrate content was 77.2 % DW, *Ulva catuca* 23.8 % DW and 20.1 % DW on *Sargassum latifolium* [13, 14, 15]. In another study reported that land plants used as raw material for bioethanol carbohydrates content on sorghum (59 % DW), sweet potato (59.3 % DW), wheat straw (67.2 % DW) and corn stover (69.7 % DW) [16, 17, 18]. The carbohydrate content in the macroalgae samples varied between red and brown. The highest carbohydrate content was obtained by *G. latifolium* (37.50 $\mu$g $(DW)^{-1}$), followed by *G. rugosa* (34.27 $\mu$g $(DW)^{-1}$) and *S. cristaefolium* (33.33 $\mu$g $(DW)^{-1}$). The red algae shown averagely higher carbohydrate content compared to brown algae. Macroalgae have various types of carbohydrates with different sugar monomers. Red algae have polysaccharides in the form of κ-carrageenan and agar consisting of various types of monomers. κ-carrageenan and agar contain sugars in the form of sulphate of D-galactose, sulphate esters of (3,6)-anhydro-D-galactose [19]. The cell wall of red algae consists of cellulose and agar so that it is easily

hydrolyzed when compared to brown algae [20]. Hence, overall red macroalge potentially exhibits the highest carbohydrate content compared to other brown or green macroalgae. However, futrther studies in a larger sample amount would be needed to confirm this.

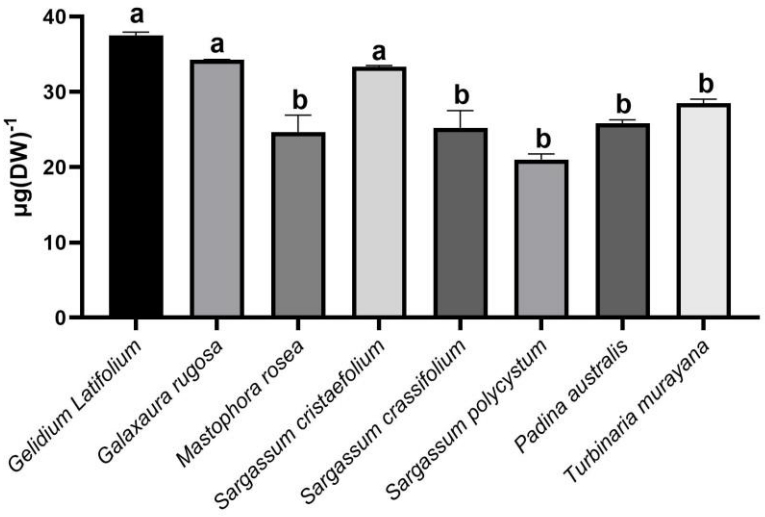


**Figure 1.** Carbohydrate total of red algae and brown algae

## 4. Conclusion

In conclusion, the highest total carbohydrate content was obtained by the red macroalgae, *G. latifolium*. The value was significantly higher compared to other macroalgae in this study. However, the overall carbohydrate content found in macroalgae was sufficient. Which concludes the potential of macroalgae as a valuable source for development of bioethanol.

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