Potential for Biodiesel Production of Selected Seaweed Species from Taklong Island, Guimaras

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Abstract – The total lipid content and fatty acid composition of *Dictyota dichotoma, Padina minor*, and *Sargassum cristaefolium* found in Taklong Island, Guimaras were evaluated for their biodiesel potential. The total lipid contents were determined using the Bligh and Dyer method while the fatty acid compositions were analyzed using Gas Chromatography-Mass Spectrometry. *Padina minor* (11.00% of its weight(w)) showed the highest total lipid content, followed by *Dictyota dichotoma* (8.00% w), and the lowest is *Sargassum cristaefolium* (5.67% w). This study evaluated the lipid content of the species and supported established researches regarding conspecific variations between species. With a total lipid content exceeding 10%, *Padina minor* may be considered as a suitable candidate for biodiesel production in terms of total lipid content. The assessment of the biodiesel properties of the three seaweed species also passed the European biodiesel standards: EN 14214 and ASTM D6751-02.

Introduction. – The world currently relies heavily on fossil fuels as an energy source providing for more than 85% of the worlds energy needs [1]. However, fossil fuels are finite and can cause harmful effects to the environment due to its excessive greenhouse gas emissions [2],hence the demand to develop alternative ways to minimize our dependency to fossil fuels has become of great importance [3].

In an effort to answer this problem, recent researches focus on the production of sustainable bioenergy resources such as biofuels. Unlike the conventional burning of fossil fuels, biofuels get its energy from biological sources, such as vegetable oil and animal fats, that can be used for heat, electricity and fuel, which, overall, produces less greenhouse gases [4]. Additionally, biofuels produce renewable energy from biological feedstocks [5]. For this reason, its feedstocks can be stored until needed and provide a liquid fuel alternative to various energy-related crisis, including fuel for transportation, which is perceived to be dependent on biofuel in the coming decades [6].

In the Philippines, climate change has posted a major concern and led to the ratification of various acts pertaining the use of biofuels as an alternative to petroleum and other crude oils [7]. The Philippines, being an agricultural country, uses and develops land crops as feedstocks for biofuel, thus raising the conflict between food security and energy sustainability [8]. Moreover, due to its geographical location, the country is oftentimes struck by typhoons and other natural disasters that impose a detrimental impact on its agricultural resources [9], such as coconut, corn, cassava and sugarcane, which are the main sources of biofuels in the Philippines [10] .

Nonetheless, the archipelagic country has a diverse set of marine resources that is left untapped. The potential of marine organisms, especially of macroalgae, to serve as feedstocks for biofuel has received global attention for its easy and low-maintenance growth and relatively high oil yield [11]. At the present time, there about 820 species of seaweeds known in the Philippines and only a few has been studied for its biofuel potential [12]. However, the main focus of research for macroalgae is their cultivation in production of oil-based products, most especially the liquid biofuel-derived biodiesel, which is used as fuel for transportation vehicles [13].

Unfortunately, the ability of an organism to produce lipid does not necessarily make it a good candidate to be used as feedstock for biodiesel. To assess the qualification of an organism for efficient biodiesel production, there are two factors that must be considered: its total lipid content and its physical and chemical properties based primarily on its fatty acid-methyl ester composition [14].

In order to determine the quality of the lipid to be extracted and its suitability for biodiesel use, transesterification, or the process of preparing the extracted lipid to

biodiesel, is used to produce fatty acids methyl esters [15], wherein compositions are identified to be able to assess the number of different carbon chains [16]. This is going to be compared from the provisions of biodiesel standards, such as American Society for Testing and Materials ASTM D6751 and EN 14214 in Europe, which contain the appropriate range of value for different properties [17]. If the calculated values are within the range set by the standards based on the properties tested, the subject lipid is suitable for commercial use.

This research aims to identify the biodiesel potential of seaweed species from Taklong Island, Guimaras, Philippines by quantifying their total lipid content and determining the physical and chemical properties of seaweeds through the quantification and evaluation of the fatty acid methyl esters compositions of the lipid extract in order to assess their biodiesel potential [18].

Method. – *Acquisition of Seaweed.* The three most abundant seaweed samples were collected at the coast of Taklong Island, Guimaras at around 15:00 to 16:00 in three different sampling sites. The seaweed species were handpicked via snorkeling at varying depths within the range of one (1) to two (2) meters. They were placed inside one-gallon Ziploc bags filled with seawater and were transported to the laboratory inside an icebox. They were then put inside a freezer with a temperature of 4°C for storage.

Species Identification. Small representative portions of each individual samples were preserved in 70% ethanol for verification purposes. The species were identified by comparing the morphological characteristics of the samples to existing photographs of the species found on Taklong Island, Guimaras. The identified species were *Dictyota dichotoma*, *Sargassum cristaefolium*, and *Padina minor*.

Cleaning of Seaweed Samples. The seaweed samples were washed with tap water to remove attached coral parts, stones, and epiphytes, and to thaw ice bits that formed around the samples from being stored in the freezer. The samples were then sun-dried for at least an hour to remove any excess moisture.

Water Quality Test. The water quality of the three sampling sites, where each seaweed species was collected, were tested using PASCO Advanced Water Quality Sensor PS-2230 package borrowed from the SRA equipment unit of Philippine Science High School - Western Visayas Campus. The parameters quantified were temperature (with Stainless Steel Temperature Probe PS-2153), conductivity (with Conductivity Electrode 10x PS-2571), pH level (with pH electrode PS-2573), and dissolved oxygen (with Optical Dissolved Oxygen Probe 003-14185). They were measured by their corresponding sensors and were done so as per the instructions written on the handbook that went with the equipment. The values were displayed on the software that was included in the equipment package. The values of the temperature for all sample sites were specifically quantified instantaneously in order to yield more accurate data, while the values of other parameters were measured, not necessarily instantaneously, from the water samples that were acquired and stored inside Ziploc bags. All the components were calibrated before each usage with the use of distilled water to clean the sensors. Another parameter, the salinity of the seawater, was also determined as a derivation from two tested components, conductivity and temperature.

Total Lipid Analysis. The procedure followed in this study was based on the total lipid extraction method of Bligh and Dyer (1959).

Homogenization. The sun-dried samples were minced using a pair of scissors as a means to pulverize them. A triple balance beam was used to measure one-gram of minced seaweeds, which was prepared in triplicates. The weighed seaweeds were placed inside test tubes and were added with one mL of chloroform and two mL of methanol resulting in a 1:2 ratio. The mixtures were vortexed for six minutes and an additional one mL of chloroform was added again and vortexed for another 30 seconds. Finally, one mL of distilled water was added and vortexed for 30 seconds resulting in a final ratio of 2:2:1 of chloroform:methanol:distilled water mixture.

Separation of Phases. The mixtures were filtered using a glass funnel and a Whatman no. 3 filter paper setup in order to obtain their liquid components. The filtrates were then transferred into another set of test tubes where the separation of phases was going to take place. In order to do that, the samples were centrifuged at 1000 rpm for five minutes at room temperature. The upper phases (methanol layer) were siphoned out using a Pasteur pipette. The lower phases (chloroform layer) were then transferred to their respective preweighed test tubes.

Evaporation. The filtrates were treated to a hot water bath using a hot plate with the temperature set at around 185°C until the remaining chloroform contents of the solutions were removed completely, leaving the surface of the base of the test tubes clad with lipids only.

Measurement of Lipids. The total weight of the remaining lipids was measured through gravimetric analysis using an electronic balance. The values obtained from the quantification of the total weight were subtracted from the weights of their respective preweighed test tubes. The differences were the corresponding total lipid contents (TLC) of the replicates. The total lipid content percentage (TLPC) in relation to its weight was calculated with the formula:

$$TLPC(\%) = \frac{TLC(g)}{Weight of Seaweed Species(g)} \qquad (1)$$

Fatty Acid Analysis- Derivatization. The samples for lipid analysis were stored in an icebox and transported to the laboratory of the College of Fisheries and Ocean Sciences department of University of the Philippines - Visayas

(UPV) in Miag-ao, Iloilo where it was subjected to pretreatment for Chromatographic analysis. The lipids undergone derivation using 14% BF_3 as reagent in order to produce Fatty Acid Methyl Esters, afterwards these lipids were to be injected in the Gas Chromatography with Mass Spectrometry (GC-MS). The process was done with the aid of the laboratory technician of UPV.

Chromatographic Analysis. The pretreated samples were injected in the Gas Chromatography with Mass Spectrometry (GC-MS) equipment for analysis of FAMEs. The complete procedure was done using GC: Clarus 600 Gas Chromatograph; MS: Clarus 600 T-Mass Spectrometer of UPV.

Total Lipid Content. Since each seaweed species was done in triplicates, the mean of the three values, as well as the standard error of mean, of the total lipid content was calculated. The obtained results served as the final total lipid content of the seaweeds (mg g-1 sd).

Biodiesel properties. Sixteen biodiesel properties were calculated using the FAME composition of each species. Fuel properties derived from FAME profiles are the following: degree of unsaturation (DU), long chain saturation factor (LCSF), cold filter plugging point (CFPP), iodine value (IV), saponification value (SV), cetane numbers 1 and 2 (CN1 and CN2), saturated fatty acids (SFAs), mono-unsaturated fatty acids (MUFA), poly-unsaturated fatty acids (PUFA), kinematic viscosity, density, higher heating value (HHV), amount of C18:3, number of double bonds (Db), and oxidation stability.

Results. – *Water Characteristics.* The seawater parameters, including conductivity, dissolved oxygen, salinity, pH level, and temperature, did not vary much across different sampling sites given their close proximity among one another. Site 2, where *Padina minor* was collected, showed the highest temperature (29.6 C°) and conductivity (52978.33 uS/cm), followed by Site 1, where Dicytota dichotoma was gathered, (28.1 C°)(50641.73 uS/cm), and the lowest is Site 3, where *Sargassum cristaefolium* was collected, with values 28.0 C° and 42344.38 uS/cm for the temperature and conductivity, respectively. The same order applies to the salinity as the values were derived from the two parameters aforementioned. In terms of pH level, Site 1 (7.3) displayed the highest value, followed by Site 2 (7.1), and the lowest was Site 3 (7.0). On the other hand, Sites 1 and 3 showed the highest amount of dissolved oxygen (6.22 mg/L) followed by Site 2 (6.18 mg/L). (See Table 1)

Total Lipids. The total lipid content as the mean of three replicates with respect to the dry weight of *Dictyota dichotoma* (DD), *Sargassum cristaefolium*(SC), and *Padina minor*(PM) are shown in Table 2. *Padina minor* (110 mg g-1 w 1.00 SE) showed the highest lipid content, followed by *Dictyota dichotoma* (80 mg g-1 w 1.00 SE), and the lowest is was *Sargassum cristaefolium* (56.7 mg g-1 w 0.88 SE). (See Table 2).

Discussion. – This study initially aimed to discover whether the species found on Taklong Island, Guimaras on the arbitrary date of its collection would serve as good feedstocks for biodiesel production by quantitatively and qualitatively assessing their total lipid content and fatty acid methyl ester profile. In addition to that, the quality of water was also examined to create a basis for cultivation technologies in the future if the species were to be proven to be viable and efficient sources of biodiesel when applied in practice. Another reason for water testing was to plot out environmental and external factors behind conspecific variations by comparing the data with other published works.

This study provides evidence that despite having the reputation of being unsuitable for oil-based byproducts due to its presumable low lipid yield, some seaweed species exceed other microalgae species [19], which are in theory viable candidates for biodiesel feedstock for its high lipid content, although their cultivation proves to be economically inefficient. In terms of lipid content alone, two of the three species can be prospected as candidates for cultivation for its relatively high lipid content: *Padina minor* (11% w) being the highest, and *Dictyota dichotoma* (8% w).

There have been no study yet done specifically on the lipid content of *Padina minor*; however, the genus Padina generally has lower lipid content, with values averaging from 1-5% and has been found to contain higher level of polyunsaturated fatty acid composition in comparison to terrestrial crops [21], which results in more unstable and less quality biofuel but fit for pharmaceutical functions and human consumption [22]. These differences between the species and genus scale of the organism therefore suggests that, rather than being of a unique genus group, *Padina minor* is more of a unique individual species by having an unusually high oil yield.

Almost contrastingly, the genus Dictyota has relatively high lipid yield, even exceeding 20% w, a value that can compete with most microalgal species [23]. Specifically, *Dictyota dichotoma* had been studied for its lipid content before, with results varying between species of different locations, but more than 10% w, still, [24], although the results do not deviate dramatically from this studys (8% w). This anomaly called conspecific variable is a natural characteristic of organisms living in different environmental conditions. This rests the case on the importance of measuring environmental factors (in our study, the water quality) that may affect the chemical compositions of an organism. Also, by studying this, and proving the relationship between an organisms genotype and its environment, people may be able to develop optimal cultivation technologies and conditions to increase lipid yield among seaweeds, which have relatively low lipid content but are low-maintenance and fast-growing. Thereby, spending less on the production of biodiesel, or any other oil-based byproducts of seaweeds for that matter. The possible effects of water quality to the species will be fur-

Site Num.	Conductivity (uS/cm)	Dissolved Oxygen (mg/L)	Salinity (PSU)	pH Level	Temperature (C°)
1	50641.73	6.22	31.06	7.3	28.1
2	52978.33	6.18	31.66	7.1	29.6
3	42344.38	6.22	25.49	7.0	28.0
Average	48654.81	6.21	29.40	7.1	28.6

Table 1: Value of seaweed parameters measured at each sample site.

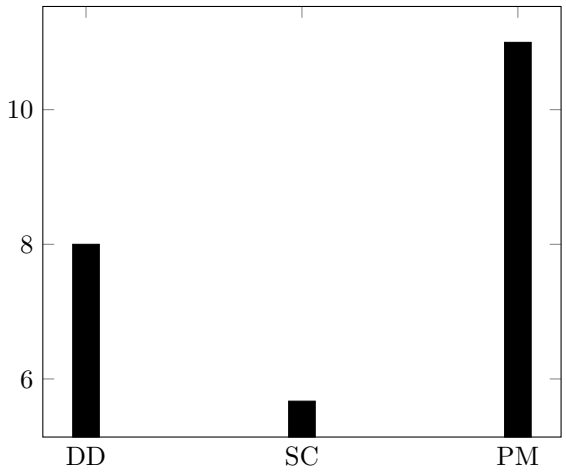

Table 2: Total lipid content (%) of the three samples

ther discussed in detail later on.

Like *Padina minor*, there is little study conducted on the lipid content of *Sargassum cristaefolium*, but a patent on the use of the species yielded slightly conclusive results with the present study [25]). In the patent, the species only contained 2% w, which suggests that *Sargassum cristaefolium* has low lipid content. However, the difference between the lipid percentage (almost 4%) is worth taking notes and implies that there could be something that had affected the species in our study to produce such a difference between the same species. This also suggests a case of conspecific variance due to difference in location. Although there does not have plenty of studies pertaining the lipid content of the genus Sargassum in general, the use of it to produce biofuel is not foreign [26].

On the other hand, several research studies observed environmental conditions to be some of the factors that influence the production of lipid in organisms. In the review study of Juneja et al [27], they noted that the factors affecting the increase in lipid growth as a survival mechanism or biological comfortability included lower pH level, lower temperature, and higher salinity. For the other two parameters, dissolved oxygen and conductivity, there are no studies focusing on their influence on the production of lipid in seaweeds; however, conductivity is related to salinity as the latter parameter is derived from the latter. In the present study, organisms of the same species were not subjected to different environmental conditions, rather the water quality of each species were gathered and assessed whether it affected the amount of lipid content or not, in comparison to other studies. As the direct mechanism with which each species practiced most could not be observed, and the values did not vary dramatically, no solid connection can be established. Also, the values are conclusive to the normal values of seawater parameters. Despite the claims of other studies regarding the relationship between the genotype of an organism and its environmental location, this study does not have enough comparisons to support the claim.

Conclusion. – This study evaluated the total lipid content and fatty acid composition of *Dictyota dichotoma*, *Sargassum cristaefolium*, and *Padina minor* for biodiesel production. Of the three species screened, *Padina minor* showed the highest potential in terms of its lipid content and it may be a suitable candidate for biodiesel production.

To further improve this study, the lipid and fatty acid content of the remaining seaweed species must be explored. To add to this, the lipid and fatty acid content should be documented throughout the year, since the environmental conditions of different seasons might affect their lipid content. By extension, other external factors should also be considered and studied, in order to attain optimal cultivation technology for higher lipid yield from seaweeds. The lipid and fatty acid content at different seaweed parts should also be taken into account. The freshly gathered seaweed samples should also be cleaned prior to storage. In determining the lipid weight, the Folch method is the better procedure since the Bligh and Dyer method loses its accuracy when extracting tissues with high lipid contents. To further increase the accuracy of the results, the sample sizes and solvent volumes should be increased to limit the possibility of anomalous data. It lowers the difficulty in separating the methanol and chloroform phases therefore decreasing the chances of having leftover methanol in your filtrate. To maximize the extract gathered, the leftover tissues should be re-extracted again using chloroform and methanol. In the evaporation of the chloroform phase, it should be noted that it should not be exposed to air as it will cause oxidation to the fatty acid content thereby affecting the data. To remedy this, it is recommended that nitrogen should be used to evaporate the chloroform phase as it limits its contact with air.

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