INNSPUB

Journal of Biodiversity and Environmental Sciences (JBES)
ISSN: 2220-6663 (Print) 2222-3045 (Online)
Vol. 16, No. 6, p. 33-44, 2020
http://www.innspub.net

RESEARCH PAPER OPEN ACCESS

Bioethanol production from non-edible macroalgae collected in the waters of STA. ANA, Cagayan using microbial fermenters

Andy L. Catulin*

Faculty of Biology, College of Arts and Sciences, Cagayan State University, Carig Campus, Tuguegarao City, Philippines

Article published on June 20, 2020

Key words: Bioethanol, Macroalgae, Fermenter, Percentage dry weight, Acid pretreatment

Abstract

The study determines the bioethanol production from selected non-edible macroalgae using different microbial fermenters. The bioethanol production included two processes; first involving acid pretreatment was carried out in this study to further degrade the complicated sugar present in macroalgae for seven (7) days. Second, anaerobic fermentation using four microbial fermenters. The result of the study showed that there were fifteen (15) species of macroalgae collected and identified. Among the fifteen non-edible macroalgae, the top ten with the highest percentage dry weight includes the three species of Halimedeae with Percentage Dry Weight (PDW) of 33 per cent, 27 percent and 24 percent for *H. macrolaba, H. opuntia* and *H. tuna* respectively. In terms of sugar content using Brix refractometer, the top five non-edible macroalgae species after pre-acid treatment were as follows: *Liagora* sp., *Galaxaura oblongata, Sargassum crasssifolium* with 3°Br; and *Turbinaria oranata* together with *Padina japonica* with 2°Br. The used of 30g/0.1kg dry weight sample among the five selected non-edible macroalgae utilized in the fermentation process yielded sufficient ethanol of 2.99 percent to 4.17 percent. Statistically, regardless of the non-edible macroalgae and microbial fermenter used in the study, there was no significant difference in their ethanol production. However, *Liagora* sp. showed the highest percentage ethanol production and the yeast microorganism *Candida tropicalis* was the best fermenter. Bioethanol from non-edible macroalgae such as the species of *Liagora* sp, *G. Oblongata, S. crassifolium, T. oranata* and *P. japonica* which were available.

***Corresponding Author:** Andy L. Catulin ✉ andy_catulin@yahoo.com

Introduction

Macroalgae which are capable of accumulating high starch/cellulose can serve as an excellent alternative to food crops for bioethanol production, green fuel for a sustainable future (Ra & Kim, 2015). Certain species of algae can produce ethanol during dark-anaerobic fermentation; thus, serve as a direct source for ethanol production (Sulfahri *et al*., 2016). Macroalgae is also harnessed as a renewable source of biomass intended for ethanol production. Currently, there are very few studies on this issue, and intense research is required in future in this area for efficient utilization of algal biomass and their industrial waste to produce environmentally friendly fuel bioethanol (Özçimen, İnan & Biernat, 2015).

Some 820 species of marine algae, including many species of Cyanophyta, are reported from the Philippines. These consist of 472 species of Rhodophyta belonging to 37 families and 11 orders, 134 species of Phaeophyta belonging to 10 families and seven orders and 214 species of Chlorophyta belonging to 11 families and 7 orders. The Rhodophyta comprise 57.6 per cent, the Phaeophyta 16.3 percent and the Chlorophyta 26.1 percent of the flora. Many of these species are of economic importance as food, sources of industrial products such as polysaccharides, bioactive and nutritional natural products, and growth promoting substances (Trono, 2004; Evangelista *et al.* 2015; Clemente, Baldia, & Cordero Jr, 2017).

The study conducted by Masirag (2013) on the macroalgae diversity, utilization and phytochemical screening resulted on the 48 prevalent species of macroalgae found in the coastal towns of Cagayan for the months of October, November and December. The macroalgae was dominated by Rhodophyceae. Thirteen (13) species belonging to Chlorophyceae, eleven (11) species belongs to Phaeophyceae, and twenty-four (24) species classified under Rhodophyceae. Further, the study also revealed that the most diverse coastal town in terms of macroalgae species found was Sta. Ana with twenty-six (26) species (Salosso & Jasmanindar, 2018; Vieira *et al*., 2017).

Macroalgae is promising bioethanol feedstock due to their fast growth rate and large biomass yield, with superior productivity to many terrestrial crops (John *et al*., 2010; Chow *et al*., 2015). The high yield of macroalgae is attributed to their lower energy requirement for the production of supporting tissues than terrestrial plants, in addition to their capability to absorb nutrients over their entire surface area, and the energy-savings derived from zero requirements for internal nutrient transport (Wi, 2009; Hamouda, Hussein, & El-Naggar, 2015.). Many types of seaweed exhibit a mass productivity of 13.1kg dry weight m-2 over a seven month growth period, compared to terrestrial plants achieving 0.5-4.4kg dry weight m-2 over an entire year. Furthermore, macroalgae generally have a greater hydrolysable carbohydrate content and potential volume of ethanol than current bioethanol feedstocks. (Ghadiryanfar *et al*., 2016)

According to the Philippine's Department of energy (DOE), the Philippines required around 219 ML of bioethanol in 2010 to comply with the 5% by volume gasoline blending mandate, as per the Biofuel Act of 2006 (RA 9367). The Act's blending rate increased to 10% (by volume) in 2011, which is expected to displace around 461 ML mineral fuel demand. By 2014, the general increase in national fuel consumption is projected to increase bioethanol demand to 536 ML annually (DOE, 2007; Le Bouthillier *et al*., 2016). As of 2009, there were only two local bioethanol producers, Leyte Agri Corp, and San Carlos Bioenergy Inc. the Leyte Agri Corp commenced bioethanol production in late 2008 , with an approximate annual production capacity of 9 ML. The San Carlos Bioenergy Inc., the larger facility of the two, commenced operation in late 2009 as an integrated sugar mill, cogeneration plant, and distillery, with an estimated annual bioethanol capacity of 30ML. in 2011, the Ethanol Producers Association of the Philippines reported that approximately 80 ML would be produced (Poquiz, 2010; Elande, & Putsche, 2018). However, these three production Figs translate to annual domestic production deficit of 170ML in 2009 and 140 ML in 2011.

Currently, the shortage of domestic bioethanol is met by the importing bioethanol from Brazil (Gatdula, 2010; Devlies, 2017). To redress the domestic deficit, the Philippine Government plans to develop a USD5 million, 100 ha bioethanol macroalgae farm in the province of Aurora in Luzon, using technology developed by the Korean Institute for Industrial Technology (Galvez, 2010; Kim *et al*., 2017).

Macroalgae represents an unrealized potential to expand existing mariculture industries, and to diversify gasoline supply from mineral fuel imports to domestic bioethanol producers in Pacific island nations (Chen *et al*., 2015). However, industrial-scale marine macroalgae culture requires significant basic research and development for species and cultivar selection, in addition to harvesting and pre-processing technology investment (Sudhakar *et al*., 2018). Furthermore, the development of efficient and cost-effective fermentation processes, and post fermentation markets for macroalgal waste biomass requires further research. The investment stimulus in the Philippines from the Biofuel Act, and the impetus to mitigate both mineral fuel and biofuel imports may provide such an incentive (Beck & Martinot, 2016). Nevertheless, it recommended to a targeted and collaborative range of initiatives focusing on each point in the supply chain from farmer to biorefinery to explore the technical and commercial potential of this new industry.

Seaweeds have a variety of used in man's everyday life. It is use as food, fodder, fertilizer, and medicine. As fodder, it is food for goats, cows, sheeps, horses, and hogs. As medicine, it is use for treatment of goiter and other glandular troubles. It is also used as vermifuges, or for the treatment of diarrhea and other glandular troubles. It is also used as vermifuge, or for the treatment of diarrhea and other stomach or urinary disorders (Mahadevan, 2015).

Seaweeds are also sources of important raw materials such as agar, carrageenan, and algin which have various uses in industries (Khalil *et al*., 2018).

These are use in thickening, suspending, stabilizing, emulsifying, gel-forming, and film-forming colloids. Algin provides ice cream its smooth texture by preventing formation of ice crystals. It is used as a suspending agent in auto polishes, in paint, in pharmaceuticals, in drugs and intibiotics (Manufacture & King, 2019). As a stabilizer agent, it serves in the processing of ruber latex and in the printing of textile. Agar, on the other hand, is used in bacteriology for the formation of the medium for the culture of bacteria. As a food adjunct, it is used as gelatin, anti-drying agent in breads and pastry, in improving the slicing quality of cheese, in the manufacture of frozen dairy products, etc. In industry, it is used for the waterproofing of paper cloth, in photographic film, agar imparts stiffness and gloss to finished leather. Carrageenan resembles agar but it has high ash content and requires higher concentration to form gels it is used I the making of surgical jellies, salves and ointments. Also, it acts as a stabilizing agent in ice cream, sherberts, and other frozen dairy products. In the Philippines, quite a number of seaweeds have economic potentials as food, medicine, fertilizer and as sources of industrially important colloids (Schiener *et al*., 2015).

As bioethanol research continues at an unprecedented rate, the development of new feedstocks and improvements in bioenergy production processes provide the key to the transformation of biomass into a global energy resource. With the twin threats of climate change and depleted fossil fuel reserves looming, it is vitally important that research communities are mobilized to fully realize the potential of bioenergy. (Muktham *et al*., 2016)

The utilization of macroalgae, particularly the non-edible ones provide a new horizon for bioethanol production. Macroalgae grows faster than terrestrial crops, have a high sugar content for conversion to advanced biofuels and ethanol, absorbs more airborne carbon than land-based plants, have no lignin, can be easily harvested compared to microalgae and other terrestrial plants, requires no pretreatment for ethanol production, can be harvested up to six times a year in warm climates (Chen *et al*., 2015).

Identification of the best non-edible species of macroalgae presents in the water of Cagayan in terms of its biomass and sugar content; and utilizing the most suitable microbial fermenter will provide a new opportunity for its utilization and development.

Generally, this study aimed to determine the bioethanol yield from selected non-edible macroalgae using different microbial fermenters. Specifically, it aimed to determine: (1) The different non-edible macroalgae species present in the coastal water of Sta. Ana, Cagayan; (a) Which among the non-edible macroalgae has the highest: Percentage Dry Weight (PDW) as a measure of its biomass, and Sugar content (Brix); (2) The bioethanol yield from the selected non-edible macroalgae using different types of microbial fermenters, namely: *Saccharomyces cerevisiae BIOTECH 2055*; *Lactobacillus brevis BIOTECH 176; Trichoderma harzanium BIOTECH 3019*; and *Candida tropicalis BIOTECH 2085; (3)* Which microbial fermenter and non-edible macroalgae produce the highest percentage ethanol content.

Materials and methods

Experimental Design and Experimental Lay-out

The treatments used were five (5) non-edible macroalgae as follows: T1 *Galaxaura oblongata*, T2 *Liagora* sp., T3 *Sargassum crassifolium,* T4 *Turbinaria oranata* and T5 *Padina japonica* using subjected to four (4) Microbial Fermenters- *Saccharomyces cerevisiae* (M_0), *Lactobacillus brevis* (M_1), *Candida tropicalis* (M_2) and *Trichoderma aureoviridae* (M_3). The different treatments were laid out following thye Completely Randomized Design (CRD) in a single factorial experiment. Each treatment was replicated three times.

Procedure

Phase 1: Collection, Identification and Classification of non-Edible Macroalgae

Materials

The following materials that were used in the study includes: labelling materials (pen, masking tape, and sticker), plastic bags (zip lock), and pair of scisssors, knife, and box and transect line.

Sampling Site

The sampling site were situated at the intertidal zone of Palaui Island, Sta. Ana, Cagayan particularly at (1) Baratubut Pt., 20 Punta Verde Cove and 3) Rakat Pt. which was identified by the Bureau of Fisheries and Aquatic Resources (BFAR) Regional Office No. 2. The sampling sites are the part of the fringe reef known as Robo reef situated on the eastern side of the island.

Collection and Identification of Macroalgae

The macroalgae used in the study were collected from the intertidal zone of Sta. Ana, Cagayan during low tide. Hand picking method will be used to collect the samples using transect lines to saturate the collection of the different species in the area. Sample of each species will be obtained (including the holdfast) from the substrate to avoid ecological damage. The macroalgae that will be collected will be placed in a Ziploc or plastic bag with enough seawater. The macroalgae will be pre identified using the seaweed guide by Trono (2019) and the use of Field Guides (Baleta & Nalleb, 2016) and Herbarium (TAHIL & LIAO, 2019). Likewise, the pre identified macroalgae will be brought to the Marine Resources Section of the Bureau of Fisheries and Aquatic Resources Regional Office No. 2 for pre-validation of the specimens collected. Final verification will be done at the National Museum, Manila.

Classifying the Macroalgae according to their Food Utilization

The collected macroalgae will be likewise classified as edible or non-edible. Non-edible macroalgae those that are not utilized as human foods while the edible macroalgae are those that can be eaten and used in the preparation of food (Wong and Cheung, 2000). The macroalgae will be identified using the book of Trono (1995) on "The Economic Importance of Macroalgae in the Philippines" and also from the Listing of Edible Macroalgae species from BFAR 2.

Phase 2: Screening of Non-Edible Macroalgae

Percentage Dry Weight (PDW) Determination

A 100 g of each of the fresh macroalgae was air dried for three (3) days.

After three days, they were placed in an artificial intelligence multipurpose dryer for 3 hours at 60^{O} Celcius. The dry weight of each of the macroalgae was recorded and the measurement of Percentage Dry Weight was computed using the formula:

$$\% \text{ PWD} = \frac{\text{fresh weight-dry weight}}{\text{Fresh weight}} \text{ X } 100$$

Determination of Sugar Content (by BRIX)

A 30 g of the non-edible macroalgae (dry weight) was blended by mixing 500ml of distilled water. The percent sugar was determined using a calibrated refractometer before the fermentation process. Two (2) drops of each of the sample were placed on the prism, and then direct readings of the measurement value from the eyepiece were then recorded. There were three readings of sugar content: before the acid pretreatment, 3 days after pretreatment and 7 days after the pretreatment. The top five (5) non-edible macroalgae with the highest sugar content were the one qualified for bioethanol fermentation. In case the measurement of the top five included six or more species, the values of their PDW was considered to get the top 5 highest.

Phase 3: Bioethanol Production

Pre-acid Treatment

Acid pretreatment was carried out in this study to further degrade the complex sugar present in macro algae. 10ml of 2M hydrochloric Acid was added in each sample. The filtrate was neutralized using 1% Sodium Hydroxide at pH 7.

Microbial Fermenters

Ethanol was made for purpose of producing biofuel through fermentation process involving yeasts and in some instances bacteria and other type of fungi. In this study the microbial fermenter were utilized:

Saccharomyces cerevisiae BIOTECH 2055- is known as top fermenting yeast. *Lactobacillus brevis* BIOTECH 1766- is a bacterium belonging to the genus Lactobacillaceae. *Candida tropicalis* BIOTECH 2085- is yeast that was used to produce bioethanol. *Trichoderma aureoviridae* BIOTECH 3019- has been especially famous for producing cellulolytic enzymes with relatively high enzymatic activity.

Preparation of Culture Media

The following culture media were initially prepared in slant agar and petri plates:

For the microbial fermenter *S. cerevisiae, T. aureoviridae* and *C. tropicalis* the culture medium used was Potato Dextrose Agar (PDA).

The components were weighed as follows:

200ml infusion from white potatoes

20 g dextrose

20 g agar

For the microbial fermenter *L. brevis*, the culture medium used was DeMan-Rugosa-and- Sharpe (MRS). The components were weighed as follows:

10 g peptone	2 g K_2HPO_4
10 g beef extract	5 g Sodium acetate
5 g yeasts extract	2 g di-Ammonium citrate
20 g glucose	0.2 g $MgSO_4$ hydrous
1 g tween	800.005 g $MnSO_4$ hydous

In each of the culture media, the components was placed together on a beaker, added with distilled water, and mixed. It was cooked using a hot plate until desired then dispensed into screw capped test tubes. It was sterilized; the media was placed into slanting position right after the opening of the autoclave, and then cooled.

Subculturing the Microbial Fermenters

Each of the culture media was inoculated of their respective microbial fermenters applying the aseptic technique using an inoculating needle.

Preparation of Inocula

Inocula of the cultures of the microbial fermenters were obtained from Philippine National Collection of Microorganism (PNCM) of the Nation Institute of Molecular Biology and Biotechnology (BIOTECH), University of the Philippines, Los Baños, Laguna. Culture broth was used in preparing the inocula.

The components and procedures of the media was the same only that agar was excluded to make a broth. The culture broth was then aseptically inoculated with the use of an inoculating loop. It was incubated for approximately 60 hours to be used on determining the optical density.

Standardization of the Inocula Using the McFarland Standard

McFarland standard are prepared by adding barium chloride to sulfuric acid in order to obtain a barium sulfate precipitate and they are used to standardize the quantity of bacteria in a liquid suspension. Using the appropriate McFarland standard.

A visual comparison of the turbidity of a bacterial suspension with the turbidity of the McFarland Standard was performed.

The turbidity of the test suspension (inoculum) was then adjusted until it matches the standard. Equal disappearance or distortion of the black bar indicates a similar turbidity (ASM Manual of Clinical Microbiology, 2007).

Optical Density Determination using Spectrophotometer

The optical density readings of all suspension will be obtained at 625mm using a calibrated spectrophotometer between the ranges 0.09 to 0.10.

Table 1. McFarland Standard with its Corresponding Cell Count Density.

McFarland Standard	Approximate Cell Count Density ($x10^8$ cells)
0.5	1.5
1.0	3.0
2.0	6.0
3.0	9.0
4.0	12.0

The optical density read for the appropriate numbers of cells for the microbial fermenter was set at 0.5 McFarland standards and will be immediately added to the substrate for fermentation.

Determination of Ethanol Yield

Steam distillation was used to concentrate the alcohol content of the sample. The distillate obtained was measured using a pycnometer to get the specific gravity and temperature of the distillate and a table of values to get the percentage purity of ethanol present in the sample.

A 50-ml volumetric flask and a 25-ml pipette were used to weigh the distilled water and the distillates was cleaned with soap and water then dried using a vacuum machine.

The weight of a 50-ml volumetric flask was obtained by an analytical balance. Distilled water was placed into the flask and its total weight will be determined. This was done to obtain the mass of distilled water as follows:

$$\text{Distilled Water} = \frac{\text{Mass of weight of distilled}}{\text{water plus flask}} - \text{weight of the flask}$$

This value was used to determine the specific gravity of the distillate. For the weight of the distillate, 100 ml of the fermented juice was disposed in kjeltec tubes and subjected to the kjeltec distiller at 15.56˚C for four (4) minutes. 100ml of the distillate was dispensed into a 100-ml volumetric flask.

This was allowed to cool to 29˚C. The flask and the distillate were weighted and the specific gravity was determined as follows:

$$\text{Specific Gravity} = \frac{\text{weight of the distillate plus flask - weight of flask}}{\text{Mass of distilled water}}$$

The percent ethanol was determined by using a table of specific. To determine the value of 29^{0}C for percent ethanol, values of 28^{0}C and 30^{0}c was used and interpolation by proportion was applied using the derived formula:

$$x = \frac{2b-(b-a)}{\text{value of } 28^{\circ}C} \times 2$$

Where: a =% ethanol

b =% ethanol value of 30˚C

x =% ethanol value of 29˚C

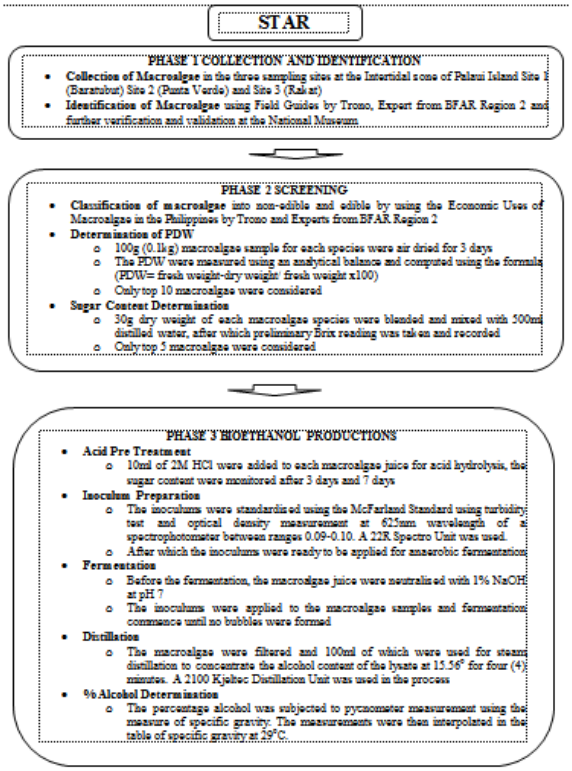

Fig. 1. General Flow Chart of the Study.

Data Analysis

The data gathered for percentage alcohol was analyzed using One Way Analysis of Variance (ANOVA). Least Significant Difference (LSD) was used to further analyze the data.

Results and discussion

Non-edible Macroalgae

Table 2 presents the list of non-edible macroalgae collected and identified from the intertidal zones of Palaui Island. There were fifteen 15 species of non-edible macroalgae collected and identified as non-edible macroalgae. The high diversity of macroalgae collected compliments the work of Masirag (2012) on the Taxonomy, Diversity and Utilization of Macroalgae in Cagayan. The macroalgae species were further classified as to their utilization for food based on the expertise of the Marine Resources Division of BFAR Region 2. Their classifications to non-edible were further verified using the work of Trono (1995) on the Macroalgae Economy and Utilization in the Philippines and Michael Guiry's Seaweed Site (2011) on Seaweeds as Human Food.

Table 2. List of non-edible macroalgae collected and intertidal zones of Palaui Island showing its family and scientific name.

Scientific Name	Family
Chlorodesmis fastiaga A. Gepp and E.S. Gepp	Udoteaceae
Udotea spinulosa M.A. Howe	Udoteaceae
Halimeda macrolaba	Halimedaceae
H. opuntia (Linnaeus) Lamourux	Halimedaceae
H. tuna (Ellis and Solander) Lamourux	Halimedaceae
Sargassum crassifolium J.G. Agardh	Sargassaceae
Turbinaria oranata (Turner) J. Agardh	Sargassaceae
Actinotrichia fragilis (Forsskal) Borgesen	Galaxaureceae
Galaxaura oblongata	Galaxaureceae
Amphiroa fragililisima (Linnaeus) Lamourux	Corallinaceae
Mastophora rosea	Corallinaceae
Liagora sp.	Liagoraceae
G. salicornia (C. Agardh) Dawson	Gracilariaceae
Hydroclathrus clathratus (C. Agardh) MA Howe	Scytosiphonaceae
Padina japonica	Dictyotaceae

Percentage Dry Weight (PDW) Determination as Measure of Biomass

Table 2 shows the percentage dry weight of the 15 non-edible macroalgae after three days of sun drying. It shows that Family Halimedacea belongs to the top five with the highest PDW of 33 percent, 27 percent and 24 percent for *H. macrolaba, H. opuntia* and *H. tuna* respectively. To complete the top ten only *Chloredesmis fastiaga* and *Udotea spinulosa* were not included registering the lowest 14 percent and 12 percent respectively. Both species belong to family Udoteacea. The top ten non-edible macroalgae with highest PDW were further subjected to sugar content determination to screen the best macroalgae species with high biomass and sugar content. Hydrolysable sugar in case of dry algae is significantly higher than that of fresh sample (Sahu *et. al.,* 2011).

Sugar Determination by Brix

Table 4 shows the sugar content of the non-edible macroalgae before and after pre-acid treatment. Degrees Brix (symbol °Bx) is the sugar content of the aqueous solution. One degree Brix is 1 gram of sugar in every 100 grams of solution and represents the strength of the solution as percentage by mass. The °Bx is traditionally used in the wine, sugar, carbonated beverage, fruit juice and honey industries (Bates, 2014).

Table 3. Percentage Dry Weight (PDW) of the Non-Edible Macroalgae as a Measure of Biomass.

Scientific Name	Average PDW/100g	Rank
Chlorodesmis fastiaga A. Gepp and E.S. Gepp	14	11
Udotea spinulosa M.A. Howe	12	12
Halimeda macrolaba	33	1
H. opuntia (Linnaeus) Lamourux	27	2
H. tuna (Ellis and Solander) Lamourux	24	4
Sargassum crassifolium J.G. Agardh	24	4
Turbinaria oranata(Turner) J. Agardh	27	2
Actinotrichia fragilis (Forsskal) Borgesen	18	7
Galaxaura oblongata	19	6
Amphiroa fragililisima (Linnaeus) Lamourux	17	8
Mastophora rosea	16	9
Liagora sp.	21	5
G. salicornia (C. Agardh) Dawson	18	7
Hydroclathrus clathratus (C. Agardh) MA Howe	15	10
Padina japonica	25	3

Before the pre-acid treatment only seven out of thirteen non-edible macroalgae shows a sugar content with a value of 1 °Bx. The species incldes; *Sargassum crassifolium, Turbinaria oranata, Galaxaura oblongata, Amphiroa fragililisima, Liagora sp., G. salicornia* and *Pandina japonica.* After three days of acid hydrolysis only *H. opuntia* did not increase in sugar content as it remains zero. T-test of significance reveals that after three days of acid pretreatment, there is a high significant difference indicating that the amount of sugar were increased by the process. After the final seven days pre-acid treatment, *s, crassifolium, G. oblongata* and *Liagora* sp. shows the highest sugar with 3°Bx each, followed by *T. oronata* and *P. japonica* with 2°Bx to complete the top five. Likewise, after 7 days acid pretreatment, there is a high significant difference before and after pretreatment. The top five macroalgae with the highest sugar content were used for ethanol production using different microbial fermenters. The higher the sugar content of the substrate the higher the ethanol conversion in the process as reported by many studies (Matsumo, 2007).

Bioethanol Yield

Table 5 shows that the five non-edible macroalgae fermented using *Saccharomyces cerevisiae* yielded ethanol ranging from 2.15 percent to 3.68 percent. *Liagora sp.* has the highest ethanol yield of 3.19% followed by *S. crassifolium* and *T. oranata* with 3.12 percent and 2.94 percent ethanol respectively. On the other hand, *G. oblongata* and *P. japonica* yielded the lowest ethanol with 2.82 percent and 2.89 percent respectively.

The mean result for ethanol yielded using *S. cerevisiae* on the five non-edible macroalgae was 2.99 percent.

Table 4. Sugar content of non-edible macroalgae in brix measurement before and after acid pretreatment with 1N HCl.

Scientific Name	Before Pre Acid Treatment	After Pre Acid Treatment (3 Days)	After Pre Acid Treatment (3 Days)	Rank
Halimeda macrolaba	0	1	1	3
H. opuntia	0	0	0	3
H. tuna	0	1	1	4
Sargassum crassifolium *	1	2	3	1*
Turbinaria oranata *	1	1	2	2*
Actinotrichia fragilis Borgesen	0	1	1	2
Galaxaura oblongata	1	2	3	1*
Amphiroa fragililisima	1	1	1	3
Mastophora rosea	0	1	1	3
Liagora sp. *	1	2	3	1*
G. salicornia	0	1	1	3
Hydroclathrus clathratus	0	1	1	3
Padina japonica *	1	2	2	2*

Analysis of variance shows non-significant difference among the treatment means This indicates that the five non-edible macroalgae shows the same ethanol yielded using *S. cerevisiae* as microbial fermenter.

Table 5. Percentage ethanol yield of five selected macroalgae using *Saccharomyces cerevisiae*

Treatment	R1	R2	R3	Total	Mean	Rank
T1 *Galaxaura oblongata*	2.77	2.64	3.05	8.46	2.82	5
T2 *Liagora sp.*	2.84	3.4	3.33	9.57	3.19	1
T3 *Sargassum crassifolium*	2.64	3.26	3.47	9.37	3.12	2
T4 *Turbinaria oranata*	2.91	2.64	3.26	8.81	2.94	3
T5 *Padina japonica*	2.84	3.68	2.15	8.67	2.89	4
Total	14	15.62	15.26	44.88	14.96	
Mean	2.8	3.12	3.05	8.98	2.99	

Table 6 shows the range of the ethanol produced from fermenting the five non-edible macroalgae using *Trichoderma aeroviridea* yielded 2.22 percent to 4.48 percent, *G. oblonagata* has the highest ethanol mean yield of 3.75 percent followed by *Liagora sp.* and *S. crassifolium* with 3.26 percent and 3.01 percent ethanol respectively. On the other hand, *T. oranata* and *P. japonica* yielded the lowest ethanol with 2.82 percent and 2.41 percent respectively.

The mean result for ethanol yield using *T. aeroviridea* on the five non-edible macroalgae was 3.05 percent, higher compared to *S. cerevisiae.* Analysis of variance shows non-significant difference among the treatment means.

This indicates that the five non-edible macroalgae shows the same ethanol yield using *T. aeroviridea* as microbial fermenter.

Table 6. Percentage ethanol yield of five selected non-edible macroalgae using *Trichoderma aureoviridea*.

Treatment	R1	R2	R3	Total	Mean	Rank
T1 *Galaxaura oblongata*	4.26	2.50	4.48	11.24	3.75	1
T2 *Liagora* sp.	3.05	3.61	3.12	9.78	3.26	2
T3 *Sargassum crassifolium*	2.64	3.33	3.05	9.02	3.01	3
T4 *Turbinaria oranata*	2.35	2.50	3.61	8.46	2.82	4
T5 *Padina japonica*	2.57	2.43	2.22	7.22	2.41	5
Total	14.87	14.37	16.48	45.72	15.24	
Mean	2.97	2.87	3.30	9.14	3.05	

Table 7 shows the range of the ethanol produced from fermenting the five non-edible macroalgae using *C. tropicalis* yielded 3.47 percent to 5.51 percent. *Liagora sp*. has the highest ethanol mean yield of 4.46 percent followed by *S. crassifolium* and *T. oranata* with 4.38 percent and 4.12 percent ethanol respectively. On the other hand, *P. japonica* and *G. oblongata* yielded the lowest ethanol with 3.80 percent and 4.07 percent respectively. The mean result for the ethanol yield using *C. tropicalis* on the five non-edible macroalgae was 4.17 percent, higher compared with *S. cerevisiae, T. aureoviridae* and *L. brevis*. Analysis of variance shows non-significant difference among the treatment means This indicates that the five non-edible macroalgae shows the same ethanol yield using *C. tropicalis* as microbial fermenter.

Table 7. Percentage ethanol yield of five selected non-edible macroalgae using *Candida tropicalis*.

Treatment	R1	R2	R3	Total	Mean	Rank
T1 *Galaxaura oblongata*	3.47	4.26	3.68	11.41	3.80	5
T2 *Liagora* sp.	5.51	3.68	4.18	13.37	4.46	1
T3 *Sargassum crassifolium*	4.26	5.06	3.83	13.15	4.38	2
T4 *Turbinaria oranata*	3.54	4.26	4.55	12.35	4.12	3
T5 *Padina japonica*	3.61	3.83	4.77	12.21	4.07	4
Total	20.39	21.09	21.01	62.49	20.83	
Mean	4.08	4.22	4.20	12.50	4.17	

Table 8 shows the range of the ethanol produced from fermenting the five non-edible macroalgae using *Lactobacillus brevis* yielded 1.07 percent to 5.73 percent. *Liagora* sp. has the highest ethanol mean yield of 5.28 percent followed by *S. crassifolium* and *G. oblongata* with 4.32 percent and 3.02 percent ethanol respectively. On the other hand, *T. oranata* and *P. japonica* yielded the lowest ethanol with 2.99 percent and 2.51 percent respectively. The mean result for the ethanol yield using *L. brevis* on the five non-edible macroalgae was 3.63 percent, higher compared with *S. cerevisiae, T. aureoviridae* but lower compared with *C. tropicalis*.

Analysis of variance shows non-significant difference among the treatment means. This indicates that the five non-edible macroalgae shows the same ethanol yield using *L. brevis* as microbial fermenter.

Table 8. Percentage ethanol yield of five selected non-edible macroalgae using *Lactobacillus brevis*.

Treatment	R1	R2	R3	Total	Mean	Rank
T1 *Galaxaura oblongata*	4.48	2.43	2.15	9.06	3.02	3
T2 *Liagora* sp.	5.73	4.69	5.43	15.85	5.28	1
T3 *Sargassum crassifolium*	2.77	4.84	3.83	12.97	4.32	2
T4 *Turbinaria oranata*	1.61	3.54	3.83	8.98	2.99	4
T5 *Padina japonica*	1.07	2.5	3.97	7.54	2.51	5
Total	15.66	18.00	20.74	54.40	18.13	
Mean	3.13	3.60	4.15	10.88	3.63	

Fig. 4 reveals the graphical summary mean percentage ethanol of the five non-edible macroalgae using different microbial fermenters, it shows that the five non-edible macroalgae yielded an ethanol ranging from 2.41 percent to 5.28 percent. Among the five selected non-edible macroalgae, the species with the highest rank on percentage ethanol yield was *Liagora* sp. with a grand mean of 4.05 percent followed by *S. crassifolium* and *G. oblongata* with 3.70 percent and 3.35 percent respectively. *P. japonica* was the slowest with 2.97 percent ethanol yield. Result of the study compliments the work of Delos Santos *et. al.* (2012) on the use of four non-edible macroalgae using *S. cerevisiae* as fermenter. Their study resulted in the production of ethanol with *Liagora* sp. registering the highest yield of 14.7 percent in a 100g sample. Generally, the result of ethanol content after the distillation process of the five non-edible macroalgae was good enough for production. The standard of economically feasible in ethanol production is only 3 percent to 5 percent (Yanagasiwa *et. al.,* 2013). Further, Analysis of Variance reveals that there is no significant difference among the treatment means of the five non-edible macroalgae using the four microbial fermenters.

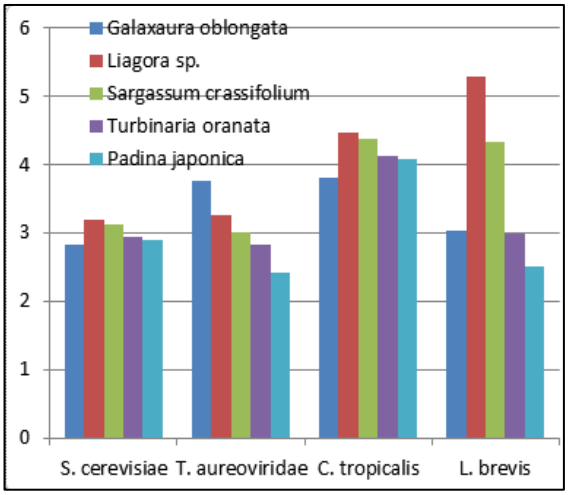

Fig. 4. Graphical Summary of Mean Percentage Ethanol of Five Selected Non-Edible Macroalgae using Different Microbial Fermenters.

Conclusions

Findings of the study revealed the following:

1. There were fifteen (15) non-edible macroalgae which includes the species of: *Halimeda macroloba, H. tuna, H. opuntia, Dicyota cervicornis, Actinotrichia fragilis, sargassum crassifolium, Galaxaura oblongata, G. salicornia Amphiroa fragililisima, Chorodesmis fastiaga, Turbinaria oranata, Udotea spinulosa, Mastophora rosea* and *Hydroclathrus clathratus*.
2. Among the fifteen non-edible macroalgae, the top ten with the highest percentage dry weight includes the three species of Halimedeae with PDW of 33 percent, 27 percent and 24 percent for *H. macroloba, H. opuntia* and *H. tuna* respectively. To complete the top ten only *Chlorodesmis fastiaga* and *Udotea spinulosa* were not included registering the lowest 14 percent and 12 percent respectively.
3. In terms of sugar content using Brix refractrometer, the top five non-edible macroalgae species after pre-acid treatment were as follows: *Liagora* sp., *Galaxaura oblongata, Sargassum crassifolium* with 3 Br, and *Turbinaria oranata* together with *Padina japonica* with 2Br.
4. Among the five selected non-edible macroalgae, the species with the highest rank on percentage ethanol yield was *Liagora* sp. with a grand mean of 4.05 percent followed by *S. crassifolium* and *G. oblongata* with 3.70 percent and 3.35 percent respectively. *P. japonica* was the lowest with 2.97 percent ethanol yield.
5. Among the four microbial fermenters, *Candida tropicalis* with a grand mean of 4.17 percent had the highest percentage ethanol yield was followed by *Lactobacillus brevis* and *Trichoderma aureoviridae* with 3.63 percent and 3.05 percent respectively. The control, *Saccharomyces cerevisiae* registered the lowest with 2.99 percent ethanol yield.
6. There was no significant difference on the percentage ethanol yield among the five non-edible macroalgae and four microbial fermenter used in the study.

Recommendations

The following recommendations are presented:

1. That the non-edible macroalgae species used in the study should be further investigated. Successful bioconversions of algal biomass to ethanol have been achieved by series of different pretreatment, hydrolysis and fermentation. Thus, the need to utilized other form of pretreatment such as physical means (steam flashing), the use of other acid solvents and improve fermentation set-up should be applied.
2. Further studies must be conducted for the production of ethanol from other non-edible macroalgae in terms of level of the use of the different fermenters influenced by time in order to evaluate the maximum efficacy to the macroalgae species with regards to ethanol production.
3. It is further recommended that the use other microbial fermenters be done for the ethanol production on macroalgae.
4. Due to the abundance of biomass as a marine resource for exploitation in the bioconversion, detailed study of hydrolysis protocol followed by fermentation need prior standardization and optimization of pH, temperature, reaction timing, and enzyme substrate concentration for better utilization of the macroalgae in the future.
5. Lastly, it was also recommended that the identified non-edible macroalgae will be subjected to DNA tagging for better identification of the species that will establish high conversion of bioethanol.

References

Baleta FN, Nalleb JP, 2016. Species composition, abundance and diversity of seaweeds along the intertidal zone of Nangaramoan, San Vicente, Sta. Ana, Cagayan, Philippines. Aquaculture, Aquarium, Conservation & Legislation **9(2),** pp.250-259.

Beck F, Martinot E. 2016. Renewable energy policies and barriers.

Chen H, Zhou D, Luo G, Zhang S, Chen J. 2015. Macroalgae for biofuels production: Progress and perspectives. Renewable and Sustainable Energy Reviews **47,** pp.427-437.

Chen J, Bai J, Li H, Chang C, Fang S. 2015. Prospects for bioethanol production from macroalgae. Trends in Renewable Energy **1(3),** pp.185-197.

Chow TJ, Su HY, Tsai TY, Chou HH, Lee TM, Chang JS. 2015. Using recombinant cyanobacterium (*Synechococcus elongatus*) with increased carbohydrate productivity as feedstock for bioethanol production via separate hydrolysis and fermentation process. Bioresource technology **184,** pp.33-41.

Clemente KJE, Baldia SF, Cordero Jr PA. 2017. The marine macroalgal flora of the Romblon Island Group (RIG), Central Philippines. Aquaculture, Aquarium, Conservation & Legislation **10(5),** pp.983-1000.

Devlies B. 2017. Ethanol as Mitigation Measure in the Transport Sector: Countervailing Perverse Effects of Uncoordinated Biofuel Standards in the US and Brazil. Energy LJ **38,** p.213.

Elander RT, Putsche VL. 2018. Ethanol from corn: technology and economics. In Handbook on Bioethanol (pp. 329-349). Routledge.

Evangelista EV, Cordero Jr, PA, Evangelista LT, Liao LM. 2015. Macrobenthic Algae of the Southern Coasts of Catanduanes Island, Philippines: Historical Account and Critical Review of Records.

Galvez M. 2010. Seaweed-based biofuel farm to rise in Aurora. The Philippine Star, Available at: http://www.philstar.com/Article.aspx?articleid=573469

Gatdula DL. 2010. DOE still undecided on ethanol import control. The Philippine Star; Available at: http://www.philstar.com/Article.aspx?articleid=584882

Ghadiryanfar M, Rosentrater KA, Keyhani A, Omid M. 2016. A review of macroalgae production, with potential applications in biofuels and bioenergy. Renewable and Sustainable Energy Reviews **54,** pp.473-481.

Hamouda RA, Hussein MH, El-Naggar NEA. 2015. Potential value of red and brown seaweed for sustainable bioethanol production. Bangladesh Journal of Botany **44(4),** pp.565-570.

John RP, Anisha GS, Nampoothiri KM, Pandey A. 2010. Micro and macroalgal 254 biomass: A renewable source for bioethanol. Bioresource Technology; DOI: 10.1016/j.biortech.2010.06.139

Khalil HPS, Lai TK, Tye YY, Rizal S, Chong EWN, Yap SW, Hamzah AA, Fazita MR, Paridah MT. 2018. A review of extractions of seaweed hydrocolloids: Properties and applications. Express Polymer Letters **12(4).**

Kim JK, Yarish C, Hwang EK, Park M, Kim Y, Kim JK, Yarish C, Hwang EK, Park M, Kim Y. 2017. Seaweed aquaculture: cultivation technologies, challenges and its ecosystem services. Algae **32(1),** pp.1-13.

Le Bouthillier Y, Cowie A, Martin P, McLeod-Kilmurray H. 2016. The law and policy of biofuels. Edward Elgar Publishing.

Mahadevan K. 2015. Seaweeds: a sustainable food source. In Seaweed sustainability (pp. 347-364). Academic Press.

Manufacture IV, King AH. 2019. Brown Seaweed Extracts (Alginates). Food Hydrocolloids **2,** p.115.

Masirag CA. 2012. Macroalgae Diversity, Utilization and Phytochemical Screening. Unpublished Dissertation Paper. Cagayan State University Graduate School, Tuguegarao City.

Muktham R, Bhargava SK, Bankupalli S, Ball AS. 2016. A review on 1st and 2nd generation bioethanol production-recent progress. Journal of Sustainable Bioenergy Systems **6(3),** pp.72-92.

Özçimen D, İnan B, Biernat K. 2015. An overview of bioethanol production from algae. In Biofuels—Status and Perspective. InTech.

Poquiz J. 2010. Ethanol producers buck biofuels law amendment. Malaya Business Insight; Available at: http://www.malaya.com.ph/07052010/busi4.html.

Ra CH, Kim SK. 2015. Bioethanol Production from Macroalgae and Microbes. Marine Bioenergy, p. 257.

Salosso Y, Jasmanindar Y. 2018. Diversity of brown macroalgae in Kupang Bay waters and alginate content potential and its phytochemistry. Aquaculture, Aquarium, Conservation & Legislation **11(3),** pp.598-605.

Schiener P, Black KD, Stanley MS, Green DH. 2015. The seasonal variation in the chemical composition of the kelp species *Laminaria digitata, Laminaria hyperborea, Saccharina latissima* and *Alaria esculenta*. Journal of Applied Phycology **27(1),** pp.363-373.

Sudhakar K, Mamat R, Samykano M, Azmi WH, Ishak WFW, Yusaf T. 2018. An overview of marine macroalgae as bioresource. Renewable and Sustainable Energy Reviews **91,** pp.165-179.

Sulfahri, Amin M, Sumitro SB, Saptasari M. 2016. Bioethanol production from algae Spirogyra hyalina using Zymomonas mobilis. Biofuels **7(6),** pp.621-626.

Tahil AA, Liao LM. 2019. *Caulerpa falcifolia* Harvey and Bailey (Chlorophyta) from Sibutu Island, Tawi-Tawi, a new record for the marine algal flora of the Philippines. Tropical Natural History **19(1),** pp.1-7.

Trono GC, Largo DB. 2019. The seaweed resources of the Philippines. Botanica Marina **62(5),** pp.483-498.

Trono GC. 2004. Field Guide and Atlas of the Seaweed Resources of the Philippines, Bureau of Agricultural Research, Department of Agriculture. Diliman, Quezon City.

Vieira C, Camacho O, Sun Z, Fredericq S, Leliaert F, Payri C, De Clerck O. 2017. Historical biogeography of the highly diverse brown seaweed Lobophora (Dictyotales, Phaeophyceae). Molecular phylogenetics and evolution **110,** pp.81-92.

Wi SG, Kim HJ, Mahadevan SA, Yang D, Bae H. 2009. The Potential value of the seaweed Ceylon moss (*Gelidium amansii*) as an alternative bioenergy resource. Bioresource Technology **100,** 6658-6660.