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Carbohydrate Determination of *Sargassum*sp. (Brown Seaweed) for Bioethanol Production

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Abstract: Generally in Myanmar, huge amounts of brown seaweeds especially, *Sargassum*spp. are cast ashore at ChaungTha Beach every summer and inhibited with recreational uses of beach. So, utilization of these seaweed could also solve environmental pollution problems. In this study, the raw material used for this work was *Sargassum*sp., member of brown seaweed. Its function is a primary building units of the framework. Total carbohydrate composition of *Sargassum*sp was determined. According to the analysis data, brown seaweed (*Sargassum* sp.) is carbohydrate rich materials for bioethanol production.

Keywords: brown seaweed, *Sargassum*sp, total chemical composition, carbohydrate

1. Introduction

Bioethanol has involved consideration as an alternative to petroleum-derived fuel. Seaweeds have been proposed as some of the most promising raw materials for bioethanol production because they have several advantages over lignocellulosic biomass. Ethanol fuel can be produced from terrestrial feedstock's that produce sugar, starch and cellulose. In addition, use of agricultural wastes and palm trees residual wastes to ethanol in tropical regions created great potential for ethanol production from wastes and
Outcomes are encouraging as alternative sources of energy [1][2]. Marine algae consist of macro algae (seaweed) and microalgae. Seaweeds are classified as green, brown and red. They are good sources of colloidal substances which are useful as gelling agents, emulsifiers and stabilizers in pharmaceutical, cosmetics and food products. Most seaweed research has dealt with chemical analysis because of seaweeds contain unique carbohydrate. *Sargassumis* ,one of the important brown seaweed that is distributed in temperate and tropical oceans of the world. It is utilized as animal feed, fertilizer and as source of alginate which has various used in industries. In Philippines, 72 *Sargassum*species have been recorded [3].The current study deals with chemical characterization of the brown seaweed, *Sargassumsp.*,a ssource of fermentable sugars for bioethanolproduction, the chemical analysis was done .

2. Materials and Methods

2.1 Raw Material

*Sargassum*sp. (Brown Seaweed)was collected from ChaungTha Beach, Ayeyarwaddy Division in Myanmar.

Figure 1 Brown Seaweed (*Sargassum* sp.) on ChaungTha Beach

2.2 Analysis of Chemical Composition

Determination of Starch Content

The ground *Sargassum* sp. sample (10 g) was prepared into a coned Whatman number 40 grade filter paper and washed four times with ethyl ether and washed four times with 70 per cent ethanol. The contents was allowed to drain and transfer the filter paper to a beaker and which added 5 cm^3 of 50 per cent (v/v) hydrochloric acid and broken up the filter paper with a glass rod. A further 10 cm^3 of the hydrochloric acid was

added in 1 cm^3 amount over thirty minutes. This solution was made up to 100 cm^3 in graduated flask using distilled water and shake for five minutes. These was filtered through a Gooch crucible and pipette off 50 cm^3 of the filtrate into a 250 cm^3 squat form beaker which contains 115 cm^3 of 96 per cent ethanol. This filtrate was stirred for one minute and washing the sides with 70 per cent ethanol and stand for five minutes. The filtrate which was decanted through a weighted Gooch crucible and washing the precipitate with 100 cm^3 70 per cent ethanol and 100 cm^3 of 96 per cent alcohol. The residue was dried with Gooch crucible for three hours at 105^0C. The percentage amount of starch content in the sample was weighted after drying.

Determination of Crude Fat Content

Five gram of sample were weighted into the thimble and was added 1-1.5 g of sand and mixed the sand and sample with a glass rod . The sample was dried in an oven at 102˚ C for 5 hours. The sample was allowed to cool in a desiccator. The thimble was inserted in a soxhlet liquid/ solid extractor. Accurately about 90 ml of ethanol was put into a clean, dry and weighted 150 ml round bottle flask. The extraction unit was assembled over either an electric heating mantle or a water bath. The solvent drips from the condenser into the sample chamber at the rate of about 6 drops per second. The extraction was continued for 6 hours. The extraction unit was removed from the heat source and was detached the extractor and condenser after 6 hour later. The flask was replaced on the heat source and evaporated off the solvent (the solvent may be distilled and recovered .). The flask was placed in an oven at 102˚ C and the contents were dried until a constant weight was reached. The flask was cooled in a desiccator and weighted the contents and the flask. The percentage of crude fat content in the sample was calculated as shown in the following.

Calculation:

$$\% \text{ crude fat} = (W2-W1) \times 100/ S$$

Where W1 = Weight of empty flask, g

W2 = Weight of flask and extracted fat, g

S = Weight of sample, g

Determination of Crude Fibre Content

The defatted sample (2 g) was weighted and added to 200 cm^3 of 1.25 per cent sulphuric acid and hold in a 400 cm^3 squat form beaker. It was essential to stir to break up any lumps. The glass rod should be tipped with a rubber 'policeman'.The beaker was needed to cover with a clock glass and needed to boil for thirty minutes. It was needed to make any loss in volume with distilled water during boiling. The hot solution was filtered through a Whatman number 54 grade filter paper and washing the residue well with distilled water. The residue was washed back in the beaker with a total 100 cm^3 of hot distilled water and then 100 cm^3 of 2.5 per cent sodium hydroxide solution was added. This procedure can be carried out using beakers which have previously been marked to indicate the volume. The residue was boiled for thirty minutes and making up any volume loss with distilled water. During this producer flute a Whatman number 54 filter paper, was placed in a weighting bottle and dried at 105˚ C for one hour and then weighted. The liquor as filtered through the weighted filter paper. Any residue was washed from the sides of the beaker (aiding the removal with the 'policeman'-tipped stirring rod), using hot distilled water, into the filter paper. There were needed to wash with hot distilled water until the washings are no longer alkaline to universal test paper. Finally, it was needed to allow draining, transferring to the weighting bottle , and drying at 105˚ C for three hours and then weighted. The percentage of crude fibre content in the sample was obtained after drying for fifteen minutes and weighted to the constant weight.

Determination of Protein Content

The ground *Sargassum* sp. sample (5 g) was weighted in a cupped filter paper. The filter paper and contents were transferred to a 300 cm^3Kjeldahl flask. Then 10 g of crystalline potassium sulphate, 0.7 g mercury (II) oxide or 0.65 g mercury and 25 cm^3 concentrated sulphuric acid were added carefully. It was needed to warm slowly and carefully to minimize frothing, then increased the heat and boiled until the solution clears, and continued the boiling for a further hour. And then it was needed to allow cooling and transferring to a 500 cm^3 flask using distilled water with carefully. The flask was connected up to a distillation unit whose outlet has been extended to dip into a 500 cm3conical flask. This flask should be contained 25 cm^3 of 0.1 M sulphuric acid containing a few drops of screened methyl red (1 part 0.2 per cent methyl red to 2 parts 0.2 per cent bromocresol green). Antibumping granules or pumice and 80 cm^3 of 50 per cent sodium hydroxide were added to the diluted digestion solution. It was regulated any tendency to forth with silicone antifoam reagent and then 1.5 g of zinc dust was added. It was needed for one to one and a half hour to distil. After this the condenser outlet was disconnected. The combined distillated and acid liquor were back titrated with 0.1 M sodium hydroxide. The equivalent amount of 0.05 M sulphuric acid was calculated that has been used in neutralizing the expelled

ammonia. The blank titration should not exceed 0.5 cm^3. The percentage of nitrogen in the sample was calculated.

Calculation:

1 cm^3 0.05 M sulphuric acid = 0.0014 g N

% protein = N × 6.25 (Where N = nitrogen)

The determination of the percentage amount protein conwas determined by multiplying the nitrogen value by the factor of 6.25.

Determination of Moisture Content

The moisture content of the Sargassum sp. (Brown Seaweed) was determined by the oven method. These involve the measurement of the weight lost due to the evaporation of water.The 2 g of sample in the petri dish was firstly weighed. The prepared sample was pre -dried into the oven and cooled in desiccators and then weighted again. The loosely cover dish was placed on metal shelf in an oven and dept at 100˚ C. The sample was dried about 4 hr in order to obtain the constant weight. The caccum line is shut off; dried air is carefully admitted in the oven. The dish was tightly covered and removed from the oven. It was cooled in desiccators 30 min and weighted again.

Calculation:

$$\% \text{ moisture} = \frac{\text{initial weight (g)} - \text{dried weight (g)}}{\text{initial weight (g)}} \times 100$$

Determination of Ash Content

The determination of the percentage amount of crude fat content in *Sargassum* sp. (Brown Seaweed) was carried out as follows.

A sample of the material was weighted into a previously ignited and weighed porcelain or platinum crucible. If a solid material was analyzed, a 2 g sample was usually weighed out. If liquid, a larger sample was used 5 to 10 g (5 to 10 ml, if the specific gravity is known) or larger, was used. Liquid samples must be dried before ignition. The sample was ignited carefully over a burner and heated until the sample was thoroughly charred. The crucible and contents were transferred to a muffle furnace and held at a dull red heat (550˚ C to 600˚ C) and the ashing process was continued until the ash has a gray-white appearance. It was needed to cool in a desiccators and obtained ash was weighted. The ash was needed to reheat in the muffle furnace for 1- hour intervals until a constant weight was obtained.

Calculation:

$$\% \text{ ash} = \frac{\text{sample weight (g)} - \text{ash weight (g)}}{\text{sample weight (g)}}$$

Determination of Carbohydrate Content

The determination of the percentage amount of carbohydrate content in *Sargassum* sp. (Brown Seaweed) was determined as follows:

Calculation:

% carbohydrate = 100- (M+ Fib + P + A + F)

Where M = moisture, Fib = fiber, P= protein,

A= ash, F = Fat

Determination of Cellulose Content

The determination of the percentage amount of cellulose content in brown seaweed (*Sargassum* sp.) was carried out as follows.

The test specimen containing 0.25 g, or less of cellulose and any amount of moisture and non-oxidizable contaminants was weighed into a 400 ml beaker containing a 2 in. stirring bar, either glass or plastic enclosed. A volume of 25.00 ml of 1.835 N potassium dichromate solutions was pipetted in and the beaker was covered with a watch glass. While the mixture was rapidly stirred, 10 ml of concentrated reagent-grade sulphuric acid (density 1.84) was slowly (15 sec) poured down the beaker spout from a graduate. After 15 seconds more, 30 ml of additional acid was rapidly added, followed 3 minutes later by 150 ml of water. The excess dichromate may be titrated immediately or at any time later with 0.5 N ferrous ammonium sulfate solutions.The volume which gives at the end-point for the above titration was recorded. This volume was used for the calculation of cellulose percent. The percentage of cellulose content in the sample was calculated as shown in the following.

Calculations:

% Cellulose = 1.24 V

Where, 1.24 is derived from 0.01240, the grams of cellulose equivalent to 1 ml of 1,835 N dichromate times 100 %. V is the ml of dichromate consumed per gram of test specimen.

Preparation of 1.835 N potassium dichromate solution

The dichromate solution contains 90.00 g per liter at 20˚ C of reagent -grade potassium dichromate dried overnight at 100-140˚ C. The solution must be filtered before use. When made up in this manner, the reagent is a standard, accurate to 0.05 % and will remain constant for years. The amounts of concentrations given in this method may vary within reasonable limits from nominal, but calculations must be based upon actual values used. These values must be known to a precision compatible with the accuracy desired in the final result. This usually requires the use of accurate or calibrated volumetric glassware.

Preparation of 0.5 N ferrous ammonium sulfate solution

The ferrous ammonium sulfate solution was made up of 195 g of ferrous ammonium sulfate and 10 ml of concentrated sulfuric acid per liter. This solution is not stable, changing slowly from day to day by air oxidation. Although this does not matter, as the titrant is always standardized against the permanent dichromate, such oxidation by air can, for convenience, be decreased by a slow uninterrupted stream of hydrogen gas, 2-3 bubbles per minute, through the solution.

3. Results and Discussion

3.1 Total Chemical Composition of Brown Seaweed (*Sargassum*sp)

Table 1 shows the experimental results of the compositions of protein, fat, fiber, ash, moisture and carbohydrate in brown seaweed (*Sargassum* sp.). Table 4.2 shows the experimental results of total carbohydrate compositions of brown seaweed (*Sargassum* sp.).Production of ethanol from brown seaweeds has received attention because of alginate, the maincarbohydrate fraction in brown seaweed, cannot beutilized directly as a substrate for ethanol production.Nonetheless, results of the present studyshowed that*Sargassum*sp. is a promising raw material for bioethanol production.

Table 1.The Compositions of Dry Brown Seaweed (*Sargassum* sp.)

Component	Test Method	Composition (wt %)
Moisture	R. Lee's Food Analysis	9.83 ± 0.56
Ash	R. Lee's Food Analysis	15.89 ± 0.27
Protein	Kjeldahl Method	7.63 ± 0.71
Crude Fiber	R. Lee's Food Analysis	11.65 ± 0.48
Crude Fat	R. Lee's Food Analysis	1.30 ± 0.52
Carbohydrate	By Difference	53.70 ± 0.16

Table 2.Total Carbohydrate Compositions of Brown Seaweed (*Sargassum* sp.)fromChaungTha Beach

Component	Test Method	Composition (wt %)
Starch	R. Lee's Food Analysis	24.62 ± 1.48
Cellulose	Heat of dilution dichromate method	3.06 ± 0.08
Alginate and Mannitol [4]	-	26.02
Total Carbohydrate		53.70

Conclusion

In Myanmar, brown seaweed (*Sargassum* sp.) is only the waste material and it can be found plenty at Rakhine and Tanintharyi costal region, Ayeyarwady Delta and Mottama costal region. If bioethanol can be produced from *Sargassum* sp. efficiently, this will be beneficial to environmental sector and energy sector. Therefore, this investigation has good potential for near future and it should be continued to get better results for bioethanol production.

Recommendation

For determination of raw composition process, the other carbohydrate composition should be determined.

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