INTERNATIONAL JOURNAL OF
ENERGY AND ENVIRONMENT

Volume 3, Issue 5, 2012 pp.761-774

Journal homepage: www.IJEE.IEEFoundation.org

The potential of macroalgae as a source of carbohydrates for use in bioethanol fermentation

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Abstract
Fossil fuel which is the global energy source gives rise to land contamination, air pollution, climate change, fuel crises, hike of price of petroleum products, crises in oil producing nations, dependency on oil producing countries and high risk associated with oil exploitation has led to a search for sustainable and efficient energy sources. Several types and sources of biofuels have been recently studied as potential source of energy to replace the environmentally unfriendly fossil fuels. Bioethanol produced from terrestrial plants have attracted the attention of the global society, though numerous controversies and debates were associated with the technology; such as the issue of food versus fuel competition, which further encouraged more research work on a sustainable renewable bioethanol source. This study aims at determining total carbohydrates from macroalgal specie (*Laminaria digitata)* for use in bioethanol fermentation, also using wet and analytical chemistry to extract and spectrophotometrically analyse the sample in respect to glucose and sucrose standards. The samples were lyophilized and the resulting powder extracted in a water bath at 100^{o}C in 15minutes. The analysis was performed using anthrone (colorimetric) method and the analyte read in a UV-visible spectrophotometer at 620nm. The result showed that carbohydrates were present in the samples, indicated by green and yellow, colourless pigments. Glucose and sucrose were the main identified sugars from the standards analysed. The concentration of sugars varied with time; months and seasons of the year. Result of the samples showed highest level of sugar concentration in May 2010 and lowest sugar concentration in November 2010. It was observed that the mass of sugars (glucose and sucrose) deposited as a result of photosynthesis, significantly contributed to the weight of biomass. The implication of the result indicated that: the smaller the biomass, the most likely it is to have lower mass of sugars and hence the less likely the potentials of synthesizing bioethanol from the sample. Conversely, a larger biomass signified the presence of more sugars, hence the greater the potentials for synthesis of bioethanol from the sample with larger biomass.

Keywords: Bioethanol; Macroalgae; Laminaria digitata; Glucose; Sucrose; Biomass; Sugars; Anthrone; Fossil fuel; Sustainable.

1. Introduction

The idea of using macroalgae biomass for energy was first conceived by Howard Wilcox in 1968 [1]. It was investigated in the United State of America and Japan as an alternative energy in 1970s after the oil crises, but was later discontinued as a result of the stability of oil prices [1]. Macroalgae are also

harnessed as a renewable source of biomass that could be used for bioethanol: [2] it has the potential for yielding more than ten tonnes per 1000m^2 per year, while CO_2 can be used for boosting the yield, hence the most favourable source for third generation biofuel due to its high carbohydrates content: it possesses high yield per growing area ranging between (10 – 150 dry tonnes / hectares /year) compared to the yield of sugarcane [1, 3]. Macroalgae in essence harnesses energy through photosynthesis. They absorb CO_2 and transform them into organic biomass which is converted to energy, like other biomass resources [3]. Macroalgae have gained tremendous research interest as an alternative source of renewable fuel due to their photosynthetic efficiencies, they are greatly considered for their natural sugars and other carbohydrates which can be fermented to bioethanol [4].

Among the species of macroalgae, *Laminaria digitata* and *Ulva lactuca* offer the best prospects, from energy perspective [3]. Scotland and Ireland have the largest resources of seaweeds whereas Norway and France are the major harvesters of the feedstock. Several initiatives have been undertaken regarding the use of macroalgae as a source of marine biomass for bioethanol production [3, 5].

The major issue associated with the first generation biofuel technology is the fact that as the production capacities of bioethanol continue to increase, the competition with agriculture for arable land use for food and biofuels will became a global issue, causing controversies and debates on the sustainability of the technology [6]. The Kyoto protocol to the United Nations Framework Conventions on Climate Change (UNFCCC), agreed in December 1997, marked an important turning point in the efforts to promote the use of renewable energy globally and to ensure that the developed countries are encouraged to decrease the net emissions of CO_2 [7]. Meanwhile the UK have been committed to the renewable transport fuel obligations (RTFO), thereby moving towards the development of transport biofuels with the target of replacing about 5.75% by volume of petroleum-based fuels. Meanwhile, recent concern over the sustainability of the technology has resulted in a re-analysis and assessment of this target which is now considered appropriate for 2014, with further targets of 10% by volume of liquid biofuels by year 2020 [6]. Global petroleum reserves will continue to diminish due to the excessive consumption of the product. The combustion of fossil fuels has created serious environmental concerns over global warming due to the increased release of greenhouse gas (GHG) emissions to the atmosphere, resulting in the search for biofuels that can be produced without large increase in the use of arable land, which macroalgae could offer [8].

1.1 Benefits and challenges

Bioethanol represents a sustainable near term renewable energy solution that has a measurable impact on the energy demand and environment. It accounts for approximately 1.5% of global transport fuels [9]. Bioethanol offers several benefits which are related to energy security, economics, and environment, it provides independence and security of energy supply. Global warming, depletion of fossil fuels and increase in price of petroleum-based fuels motivated the search for alternative, sustainable, renewable, efficient and cost effective energy source with less green house gas (GHG) emissions. Bioethanol is an excellent alternative energy source that can meet the present and future demand of fuels. It can be blended with petrol or burnt in its pure form within modified spark-ignition engines. In comparison with gasolines, a litre of bioethanol has a higher octane rating; when mixed with gasoline for transportation purposes improves the performance of petrol. Bioethanol could as well improve the fuel combustion in vehicles, thereby reducing emissions of carbon monoxide, and unburned hydrocarbons [9].

Another impressing advantages of bioethanol technology is that it contains only a trace amount of sulfur unlike the gasoline, it helps to reduce the fuel sulfur content and lower the emission of sulfur oxide, a major component of acid rain and carcinogen [9]. The urgent demand for an alternative and sustainable fuels feedstocks has emerged to replace food-based feedstocks, " The macroalgae biomass" an interesting option which can provide high-yielding source of bioethanol without compromising food supplies, rainforest or arable land use. Several macroalgae species with high sugar content have been tested for bioethanol production, which is basically one of the objectives of this study [9].

The increasing demand for bioethanol will create new opportunities. This paper is on the potential of macroalgae as a source for production of bioethanol, an environmental friendly and renewable transport fuel [2].

2. Materials and methods

Monthly samples of *Laminaria digitata, Laminaria hyperborean, Ascophilum nodosum, Fucus serratus, Fucus vesiculosis and Palmaria palmatum* were collected from Boulmer beach in Northumberland. The

sampling dates corresponded as closely as practicable to the lowest (spring) tide of the month. The samples were rinsed with filtered seawater at the Dove marine laboratory, Newcastle University, to remove the mud, sand and other attached epiphytes before stored overnight at 0 - 4^0C, they were further rinsed with tap water to detach grazing gastropods and other marine animals' .The materials were allowed to drip-dry for 20 minutes to remove surface water, The samples were spun in a salad spinner for 1 minute (OXO Goodgrips). Subsamples of approximately 4 x 20g were taken from each sample before freezing and lyophilised through freeze-drier to provide percentage dry weights. In the case of *Laminaria hyperborean*, a mixture of 20: 20g blade and stipe were mixed, where the stipe was shattered using a rubber mallet.

Additionally approximately 500g from each sample was roughly chopped and frozen at -18^0C in aluminium trays. In the case of *Laminarian hyperborean*, 500g of the blade and stipe were frozen separately. The *laminaria digitata* collected from the Boulmer beach has a short (< 10 cm) the stipe and blade were mixed before freezing. The samples were ground with blender as well as pestle and mortar, while the sieving was carried out in a 250μm mesh, it was weighed and the resulting powder (10mg) mixed with 5ml of milli Q water (merk millipore) and extracted in a water bath for four hours, at 80^0C. However, prior to analysis, 500µl of the extracts was transferred into vials using micro pipette and stored in a refrigerator at 18^0 F.

The total sugars in the samples were determined by Anthrone (Colorimetric) method. It is a non-stoichiometric method; therefore simple calibration curves were prepared by using series of standards of known concentrations as; 0, 20 40, 60, 80 and 100. 0.05g of D-(+)-glucose (Sigma Aldrich) was measured into a volumetric flask containing 500ml of milli Q water (Merk millipore). A 75% H_2SO_4 is prepared and allowed to cool in the ice bucket for at least four hours before the reaction occurred and later stored in an acid cupboard. Also, 0.002g/ml anthrone was added into a 75% H_2SO_4 containing 4ml of ethanol and gently dissolved in a magnetic stirrer hot plate (Stuart scientific). The 0.5ml of 75% H_2SO_4 and 1.0ml of anthrone reagent ($C_{14}H_{10}O$) (Sigma) were dispensed into 0.25ml sample with a dilution of 9.8ml of Milli Q water; It was capped and vortex briefly to mix, before boiled in a heating block at 100^oC until the reaction was completed in 15minutes, the solution was allowed to cool down at room temperature. A sufficient amount of the acid-heat digested samples were transferred into a semi-micro cuvettes , it was auto-zero and the records of the values of absorbance for both the standards and the samples (known and the unknown modes) taken, using UV-visible spectrophotometer (Varian, Inc.) at 620 nm as shown in Figure 1. For the sucrose standard: 0.5 of 75% H_2SO_4 and 1.0ml of anthrone with 1.04g and 0.05g of ethanol and sucrose standard respectively were used to carry out the assay.

The solvent is being held by a reservoir (known as the mobile phase). A high pressure pump (the solvent manager) is used to generate and meter a specified flow rate of the mobile phase which is typically in millimetres per minute. The sample is injected into the continuous flowing mobile phase stream by an injector (sample manager), which carries the sample into the HPLC column known as the stationary phase. The compound bands were identified and separated by a detector; the mobile phase is sent to the waste or collected as desired. The presence of the compounds are detected by an appropriate detector which is sent to its corresponding electrical signal to a computer data station.

Meanwhile, the choice of the detector is based upon the characteristics and concentrations of the seperated and analysed compounds. The representation of the seperation which chemically occured in the HPLC system is known as the chromatogram, at this point series of peaks rises from the baseline on a time line axis.

3. Results and discussion

Figure 1 show the result of the analysis of the glucose standard. The green colorations as observed in the solutions in the curvettes (Figure 1) indicates the presence of glucose as a result of the sugar reactions under acidic conditions, while the yellow colour represents other compounds.

The actual values of absorbance were obtained from the difference between the absorbance reading of the standard sample and the blank sample using the UV-visible spectrophotometer. Mean values of absorbance were then calculated. A plot of mean values of the absorbance (nm) against the concentration (mg/l) of the standard gave a straight line curve which signified a linear relationship between the average absorbance and the concentration of the standard sugar as shown in Figure 2.

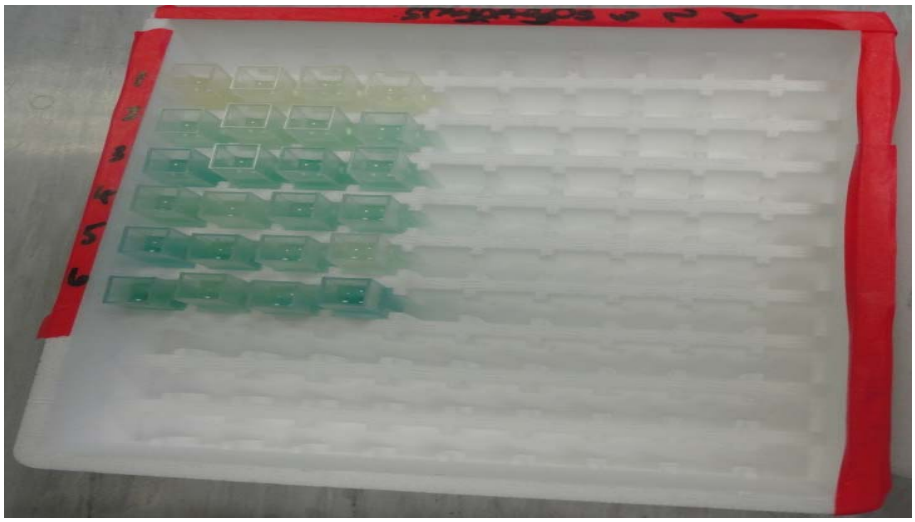

Figure 1. Glucose standard samples showing presence of glucose

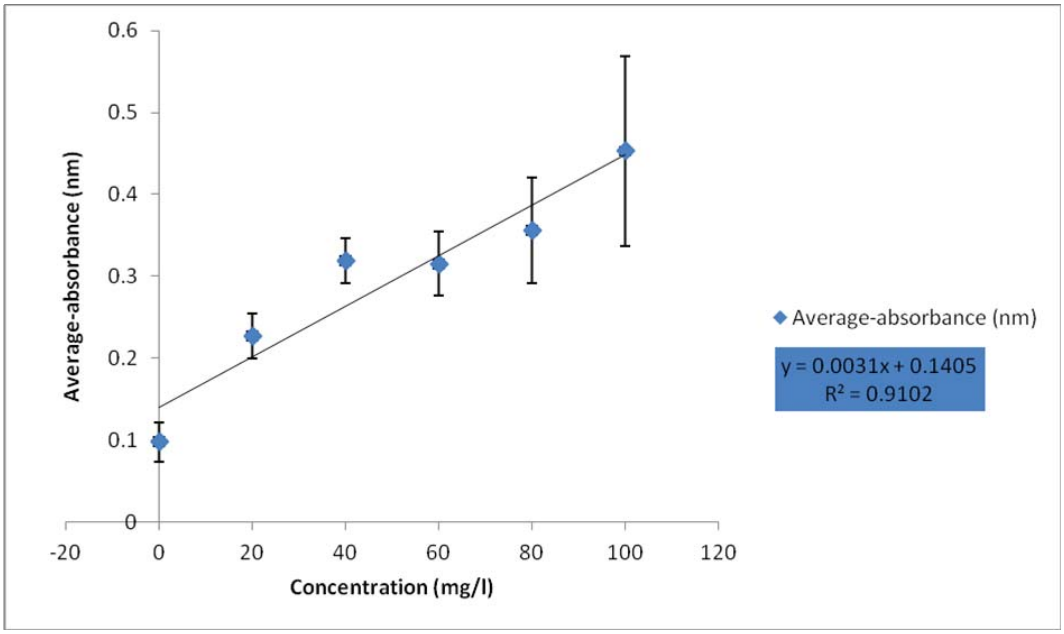

Figure 2. Glucose Standard curve showing linear relationship between average-absorbance and concentration

As shown in Figure 2, the glucose standard curve illustrates a relationship between the average-absorbance and the concentration of the standard samples. Based on the observations from Figure 2, the average-absorbance of the samples increases as concentration increases, at a wavelength of 620nm with a high correlation of 0.910. Average absorbance of 0.098 nm was observed at 0 mg/l whereas the absorbance value increased to 0.453 nm with increase in concentration to a value of 100 mg/l. The variability of the analysis was measured as a function of standard error mean which was observed to be relatively high; this was due to certain factors considered to have affected the assay such as human and instrumental errors.

3.1 Results of sample (Laminaria digitata) analysis

Results of sample reactions with anthrone under acidic conditions indicated several colourations which comprise of green, yellow and colourless solutions as observed in Figure 3. These colours signify the presence of different carbohydrate types in the sample which some of them could not be identified due to the type of equipment used in this analysis.

Figure 3. Samples of *Laminaria digitata* showing presence of different carbohydrate types

The green solutions indicate the presence of glucose, while the yellow and colourless solutions probably signify the presence of laminarin, mannitol, and other carbohydrate types as well as the presence of anthrone and sulphuric acids. The individual carbohydrate types could not be identified individually due to the insensitivity of the equipment (UV-visible spectrophotometer) used for the assay.
The concentrations of the sugars in the samples (*Laminaria digitata*), were calculated from the equation of the line of the glucose standard curve (Figure 2), where **y** designated the actual values of the absorbance and **x** the concentrations of the samples to be determined.

3.2 Results of sucrose standard analysis
Similar to results of the glucose standard analysis, pigment colourations were also observed from the sucrose standard analysis. Figure 4 shows that the green colour in the standard sucrose solution indicate the presence of sucrose sugars.
In Figure 5, a linear relationship between the average-absorbance (nm) and the sugar concentrations (mg/l) was established which signified that the rate of absorbance of light at 620nm increased with increase in concentration of the samples. A perfect linear curve that passed through the origin was obtained with the correlation factor (R^2) = 0.998 ~1, which indicated accuracy of the assay. The standard error-mean which are very small shows the accuracy of the measurements of the assay (Figure 5).

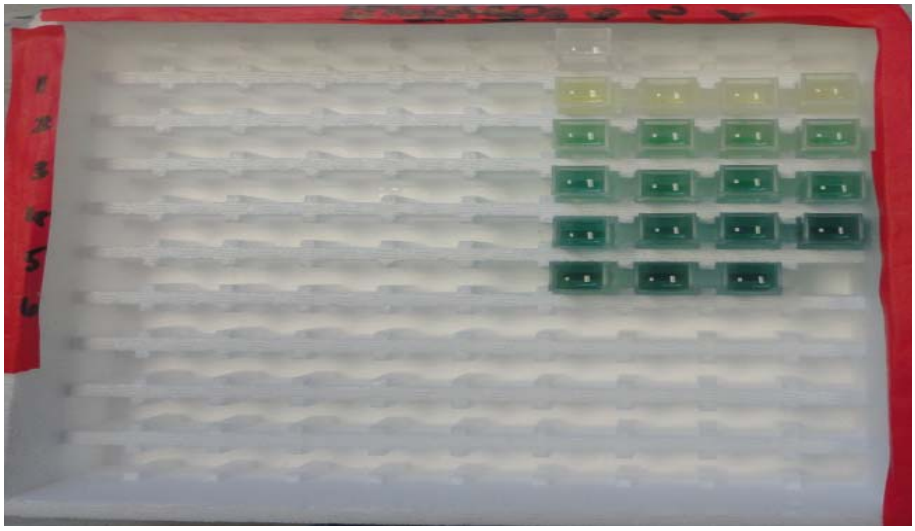

Figure 4. Sucrose standard samples showing presence of sucrose

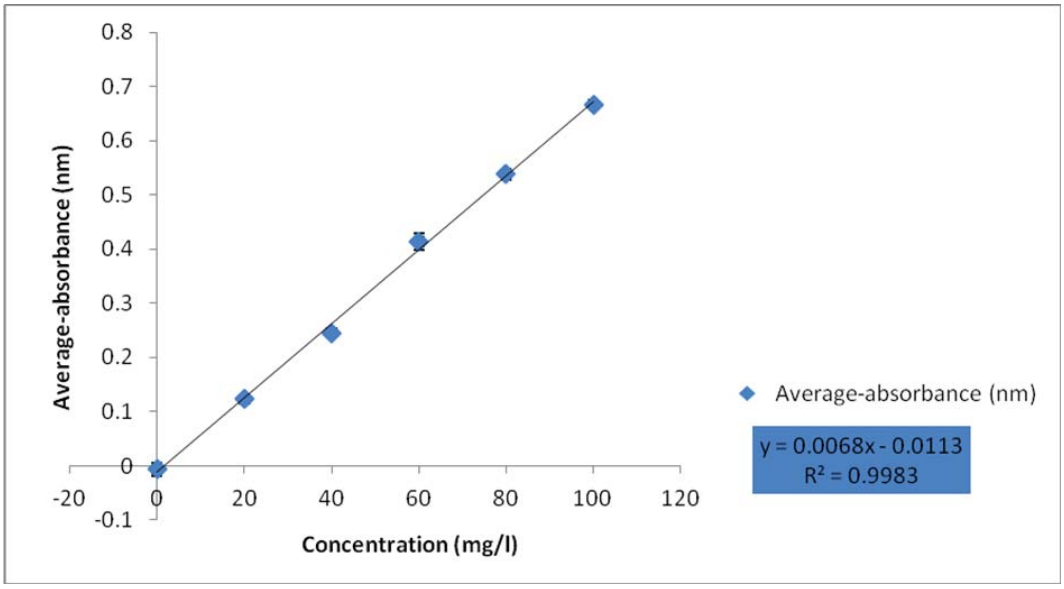

Figure 5. Sucrose standard curve showing linear relationship between average-absorbance and concentration

Table 1 below presents results of the monthly variation of sugars in the samples analysed. The results shows that the concentration of sugars (glucose and sucrose) varied at different months of the year. There was an initial decrease in average sugar concentration between July 2009 and October 2009 for glucose whereas an increase in sucrose within the same period was observed. A 61% decrease in sugar concentration for glucose and 91% increase for sucrose sugar were observed. This result indicates that a greater amount of sucrose was produced relative to glucose between the months of July and October 2009.

The glucose concentration increased rapidly from 15.9 mg/l in October 2009 to 75.1 mg/l in November 2009 (Figure 6), indicating a 78.8% increase in the concentration of glucose produced within that period. Similarly, the concentration of sucrose produced continued to increase with about 50.1% between October 2009 and November 2009. A decrease in sugar concentration was observed for both glucose and sucrose between the months of November 2009 and January 2010. While the concentration of glucose decreased by 53.9%, that of sucrose decreased by 34.2% in those months.

Maximum levels of glucose of about 152.873mg/l and 197.917mg/l were obtained for April 2010 and May 2010. Also maximum sucrose concentration was obtained in the same months with concentration of 100.122mg/l and 122.943mg/l respectively. Total sugar concentrations rose from 73.4 mg/l in January 2010 to 252.995mg/l in April 2010 and to 320.86mg/l in May 2010 (Figures 6, 7). The maximum glucose and sucrose concentrations obtained in April 2010 and May 2010 represents a 67.7 % increase for glucose from the concentration obtained in March 2009 and a 56.5 % increase for sucrose.

The monthly variation of sugars in the samples relative to glucose as shown in Figure 6 further indicates that there was consistent sugar production from January 2010 to June 2010. This may be as a result of the availability of the requirements for carbohydrate formation. The maximum values of glucose of about 152.873mg/l and 197.917mg/l obtained for April 2010 and May 2010 and maximum values of sucrose of about 100.122mg/l and 122.943mg/l (Table 1) in April 2010 and May 2010 further confirms that these months had adequate rainfall and sunshine which enhanced the growth of plant cells and carbohydrate yield of the plant through photosynthesis.

Subsequent loss of sugar concentrations was observed between July 2010 to September 2010 with the amount of -17.544mg/l, -3.081mg/l, and -5.208mg/l respectively (Figure 6). These variations could be a possible result of high intensive sunshine experienced during these periods resulting in reverse plant growth, withering the plants and decrease the yield of carbohydrate compositions in the samples (*Laminaria digitata*). The continual decrease in sugar concentration observed from November 2010 till May 2011further suggest that the losses in sugar concentration expressed as negative sugar have occurred mainly due to the high dilution of the sample during the period of the assay thereby causing low absorbance of light through the samples. It was also observed that the sugar concentration in the sample increased by 42.851mg/l in June 2011 following return of favourable conditions.

Table 1. The monthly variation of sugar concentrations of the samples relative to glucose and sucrose

Time (month)	Average-Absorbance of sample (nm)	Concentration of glucose in the sample (mg/l)	Concentration of Sucrose in the sample (mg/l)	Total concentration of sugars in the sample (mg/l)
Jul-09	0.264	41.322	2.566	43.888
Aug-09	0	0	0	0
Sep-09	-0.049	-63.057	-9.5	-72.557
Oct-09	0.188	15.911	29.456	45.367
Nov-09	0.365	75.089	59.045	134.134
Dec-09	0.138	-0.557	21.222	20.665
Jan-10	0.244	34.611	38.806	73.417
Feb-10	0.427	95.689	69.345	165.034
Mar-10	0.332	63.9	53.45	117.35
Apr-10	0.612	152.873	100.122	252.995
May-10	0.749	197.917	122.943	320.86
Jun-10	0.267	39.309	42.583	81.892
Jul-10	0.094	-17.544	13.778	-3.766
Aug-10	0.138	-3.081	21.106	18.025
Sep-10	0.131	-5.208	20.028	14.82
Oct-10	0.202	18.103	31.839	49.942
Nov-10	0.108	-12.851	16.156	3.305
Dec-10	0.243	31.502	38.628	70.13
Jan-11	0.075	-23.849	10.583	-13.266
Feb-11	0.198	16.765	31.161	47.926
Mar-11	0.1	-15.318	14.906	-0.412
Apr-11	0.129	-5.757	19.75	13.993
May-11	0.078	-22.732	11.15	-11.582
Jun-11	0.278	42.851	44.378	87.229

The result presented in Figure 7 shows the monthly variation of sucrose concentrations in the samples. The result shows that the concentration of sucrose in the sample increased in July, 2009 by 2.566 mg/l while in August 2009 no sample collection was made. Absence of sugars of about -9.5 mg/l (Table 1) was predominantly observed in September which was due to the excess dilution of the sample during the experimental work. The amount of sucrose in the sample was observed to have increased in November 2009 by 59.045mg/l (Table 1). The sugar concentrations continued increasing progressively from December 2009 till February 2010 with sucrose concentration of 21.222mg/l, 38.806mg/l and 69.345mg/l, while the sample collections made from March to May 2010, for the various amount of sucrose concentrations were observed to increase by 53.45mg/l, 100.122mg/l and 122.943mg/l (Table 1) respectively. Similar to the result presented in Figure 6, it was observed that the maximum amount of the sugar from the sample was in May, 2010 when the yield of carbohydrate compositions were enhanced by the favourable conditions of the period which is associated with adequate rainfall, sunshine and longer days. The sugar concentrations further decreased in July 2010 to 13.778mg/l while between October to December 2010, and in February 2011the concentrations were subsequently increased to about 31.839mg/l, 16.156mg/l and 31.161mg/l (Figure 7) whereas around June 2011 the sugar concentration was observed to rapidly increase to about 44.378mg/l (Figure 7) .

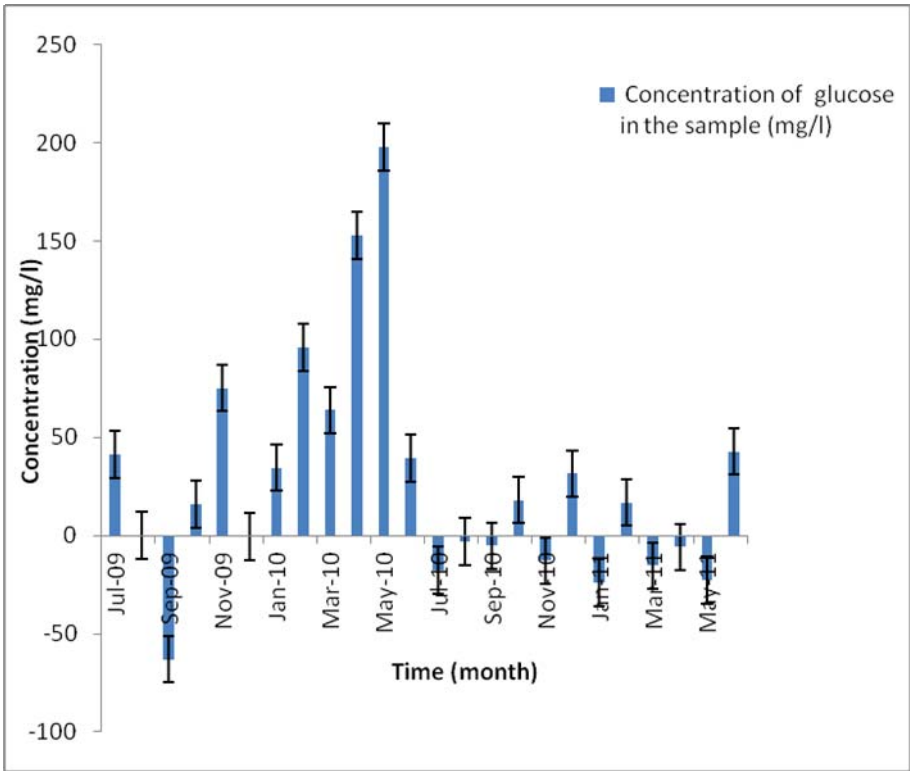

Figure 6. Monthly variations of glucose concentration in samples (*Laminaria digitata)*

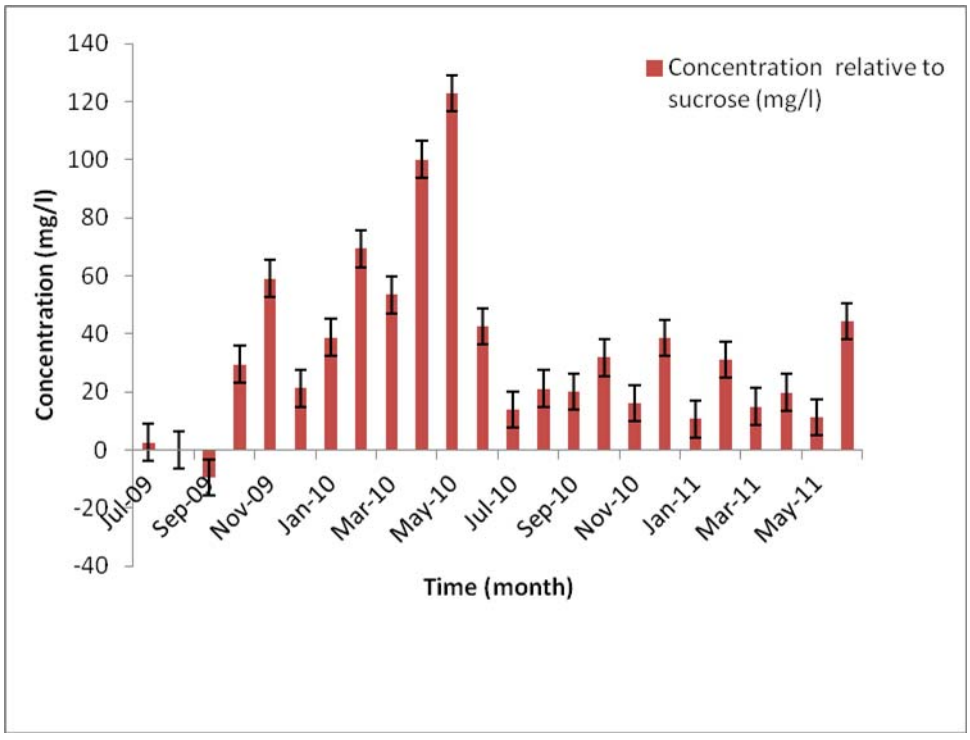

Figure 7. Monthly variations of sucrose sugar concentration in the samples

In comparing the results of the amount of glucose and sucrose in the samples (Figure 8); similar monthly variations of sugar concentrations were observed in the samples, the result indicated that in July 2009 an increase in glucose concentration was about 41.322mg/l compared to 2.566mg/l concentration of sucrose. The result shown in August 2009 indicated that no record of either glucose or sucrose were observed, this was due to no sample collection was made at the period. In September 2009; -63.057mg/l of glucose

and -9.5mg/ml of sucrose were observed in the sample, these values were attributed to high dilution of the sample during the experiment. By November 2009 the amount of sugars in the samples increased to about 75.089mg/l and 59.045mg/l relative to glucose and sucrose respectively, where the amount of glucose in the sample is much higher compared to the sucrose. In December 2009, the sample was observed to have 21.222mg/l of sucrose whereas no presence of glucose was observed. From January to May, 2010; the concentrations of both sugars were observed to increase progressively, with January recording 38.806mg/l of sucrose compare to the concentration of glucose of about 34.611mg/l.

The result also indicated that in February 2010 the amount of glucose in the sample was greater (95.689mg/l) compared to the concentration of sucrose 69.345mg/l (Figure 8). Higher concentration of these sugars were observed to be at it maximum levels between April and May, 2010; although the peak concentration of the sugars were observed in May with 197.917mg/l concentration of glucose compared to the concentration of sucrose of about 122.943mg/l whereas 152.873mg/l and 100.122mg/l (Figure 8) of glucose and sucrose respectively were observed in April. The result also indicated subsequent increases in sugar concentrations between October 2010, December 2010 and in February 2011 with about 18.103mg/l and 31.839mg/l, 31.502 and 38.628mg/l as well as 16.765mg/l and 31.161mg/l (Table 1) in both glucose and sucrose standards respectively, when greater concentration of sugars was observed in November 2009. The sugars progressively increased around June 2011 by 42.851mg/l and 44.378mg/l (Table 1) relative to glucose and sucrose respectively. From July 2010 to May 2011, a rapid decrease in both sugar concentrations were observed in the sample while absence of glucose concentrations were subsequently observed in July, August, September and November 2010 as well as in January, March, April, and May 2011 with negative values (Figure 1) which signify the occurrences of both technical and human errors or as a result of high dilution of the samples during the assay.

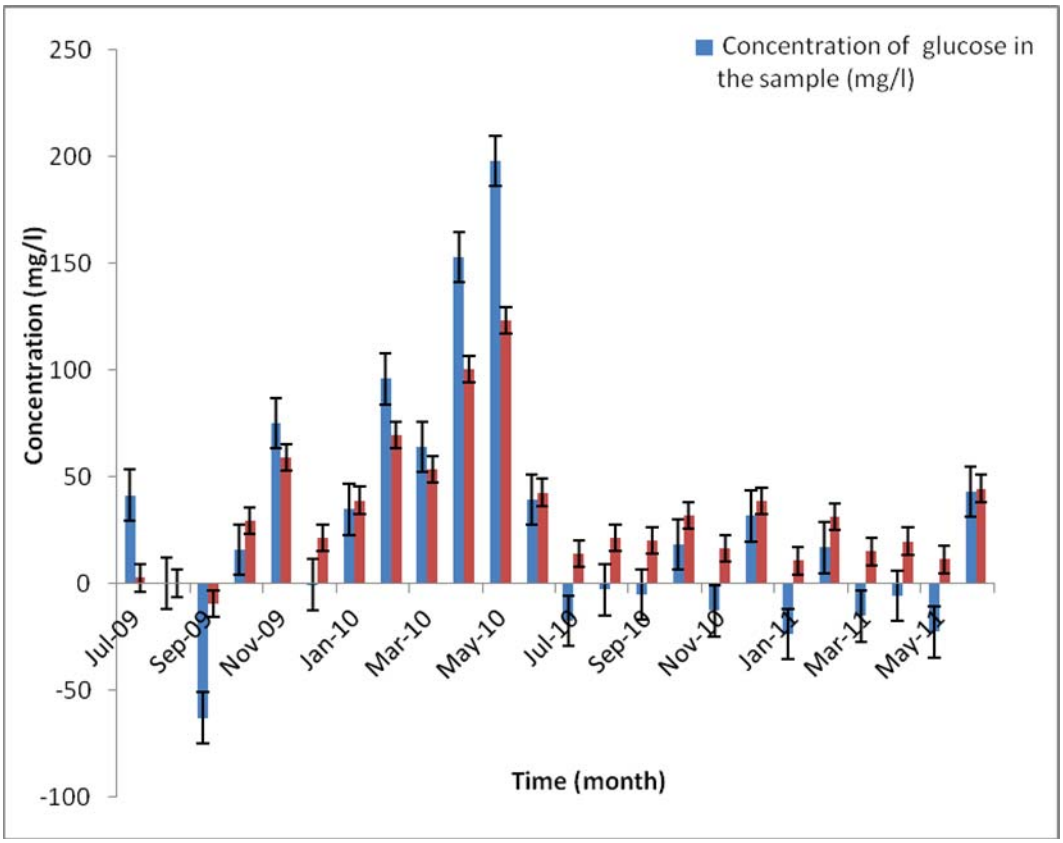

Figure 8. A graph showing a comparison of the monthly variations of sucrose and glucose concentrations in the samples

Figure 9 shows the monthly total concentrations of sugars in the sample whilst Ttable 2 presents the results of the seasonal variation of sugars in the samples.

In 2009 summer season, the average variations of 20.661mg/l and 1.283mg/l of glucose and sucrose sugars respectively in the samples gave a total sugar concentration of about 21.940mg/l. At the autumn

season about 9.314mg/l and 26.334mg/l of glucose and sucrose sugars were obtained while the total sugars in the samples during this period increased to 35.648mg/l.

During 2009/2010 winter season, approximately equal proportion of glucose and sucrose sugars in the samples of about 43.248 and 43.124mg/l gave total sugar concentration of 86.372mg/l. This could probably be as a result of mild winter conditions which favoured the photosynthetic reactions and therefore enhanced the yield of carbohydrate composition in the sample.

In 2010 spring season, high sugar concentration of about 138.230mg/l and 92.172mg/l of the glucose and sucrose in the sample resulted to a maximum total sugar concentration of 230.401mg/l. The result of this season showed a deviation from other people's work; such as study by Jessica Adams (2011) where it was observed that the highest amount of sugars in *Laminaria digitata* is present during mid-summer (July), although her work did not specify the result of other seasonal variations. Also between 2010 summer and autumn seasons, a decrease of about 32.050mg/l to 22.689mg/l sugar concentrations in the sample were observed respectively.

Further variations of sugar concentration in the sample was shown in 2011 spring time where the lowest sugar concentration of about 0.666mg/l was observed. This could probably be attributed to the intensity of the factors associated with the season which might be due to the prolonged duration of the previous winter season, such as the absence of sunshine, low rainfall, snowfalls and shorter days which would affect rate of photosynthesis in the season could be the effect of the decrease. This result therefore indicated that the amount of sugar in the samples varies with the seasons of the year, though based on the result of this study; it signifies that the samples collected during the spring time has the highest sugar concentration making it the prefered period for harvesting *Laminaria digitata* for bioethanol fermentation.

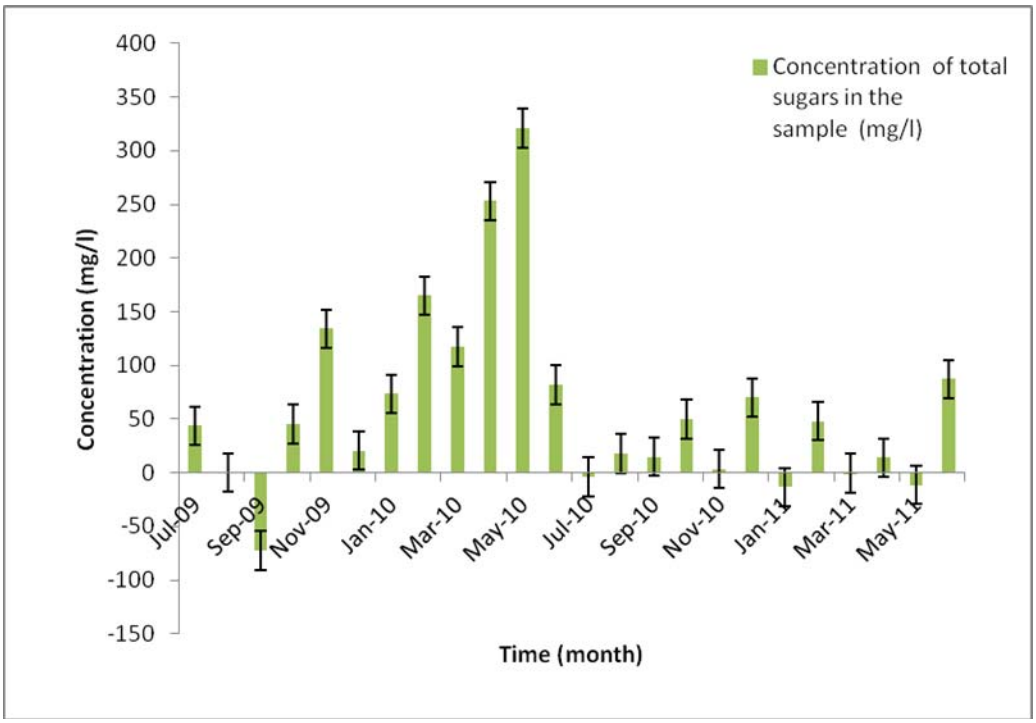

Figure 9. Result showing the total concentration of sugars in the sample

The result in Figure 10 shows an increase of 20.66mg/l and a rapid decrease of about 1.283mg/l sugar concentration in the sample relative to glucose and sucrose, a total sugar concentration of 21.940mg/l (Table 2) was also observed in the sample. In 2009 autumn season, a total sugar concentration of 35.648mg/l was obtained from the individual sugars of about 9.314mg/ml and 26.334mg/l. It was observed in 2010 winter season that equal amount of sugars of about 43.124mg/l and 43.248mg/ml relative to glucose and sucrose respectively, yields a total sugar concentration of 86.372mg/l. The increase in sugar concentration of 92.172 mg/l and 138.230 mg/l was observed in the sample relative to glucose and sucrose, from these results a maximum total sugar of about 230.40 mg/l was observed in 2010 spring season.

Table 2. Data showing the seasonal variations of the total sugars in the sample

Seasons of the year	Time (month)	Average Absorbance (nm)	Glucose concentration in the sample (mg/l)	Sucrose concentration in the sample (mg/l)	Total concentration of sugars in the sample. (mg/l)	Average seasonal concentration of glucose in the sample (mg/l)	Average seasonal concentration of sucrose in the sample (mg/l)	Average seasonal concentration of total sugar in the Sample (mg/l)
Summer	Jul-09	0.264	41.322	2.566	43.888	20.661	1.283	21.940
	Aug-09	0	0	0	0			
Autumn	Sep-09	-0.049	-63.057	-9.5	-72.557	9.314	26.334	35.648
	Oct-09	0.188	15.911	29.456	45.367			
	Nov-09	0.365	75.089	59.045	134.134			
Winter	Dec-09	0.138	-0.557	21.222	20.665	43.248	43.124	86.372
	Jan-10	0.244	34.611	38.806	73.417			
	Feb-10	0.427	95.689	69.345	165.034			
Spring	Mar-10	0.332	63.9	53.45	117.35	138.230	92.172	230.401
	Apr-10	0.612	152.873	100.122	252.995			
	May-10	0.749	197.917	122.943	320.86			
Summer	Jun-10	0.267	39.309	42.583	81.892	6.228	25.822	32.050
	Jul-10	0.094	-17.544	13.778	-3.766			
	Aug-10	0.138	-3.081	21.106	18.025			
Autumn	Sep-10	0.131	-5.208	20.028	14.82	0.0147	22.674	22.689
	Oct-10	0.202	18.103	31.839	49.942			
	Nov-10	0.108	-12.851	16.156	3.305			
Winter	Dec-10	0.243	31.502	38.628	70.13	8.139	26.791	34.93
	Jan-11	0.075	-23.849	10.583	-13.266			
	Feb-11	0.198	16.765	31.161	47.926			
Spring	Mar-11	0.1	-15.318	14.906	-0.412	-14.602	15.268	0.666
	Apr-11	0.129	-5.757	19.75	13.993			
	May-11	0.078	-22.732	11.15	-11.582			
Summer	Jun-11	0.278	42.851	44.378	87.229	42.851	44.378	87.229

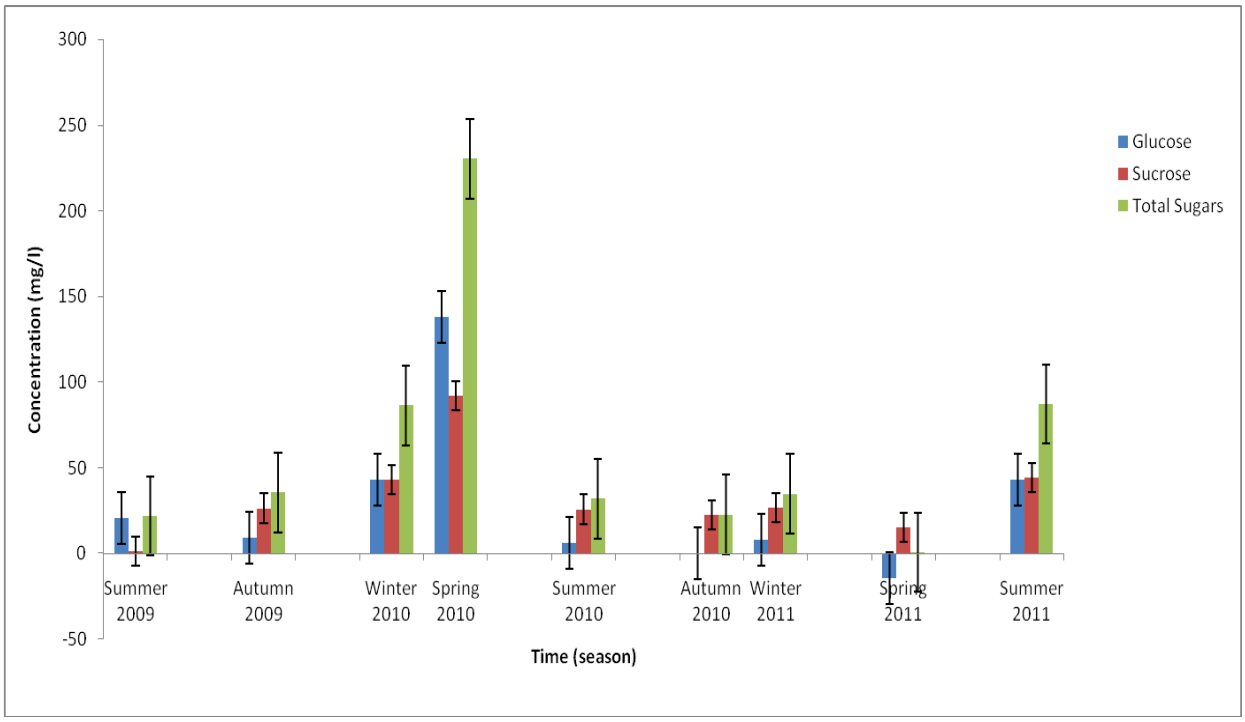

Figure 10. Result showing the seasonal variations of sugar concentrations in the sample (*Laminaria digitata*)

Further variation in sugar concentrations was observed between the result of the sample collected in 2009 and 2010 summer season. The result showed that the amount of sugars relative to glucose varied with

6.228 mg/l compared to the amount of sugars obtained in 2009 which is higher at 20.661mg/l, although the concentration of sugars at 25.822 mg/l was observed with a total sugar of 32.050 mg/l. These variation was also observed during the autumn season were a very little amount of sugars of about 0.01477mg/l relative to glucose was observed in the sample while about 22.674mg/l of sugars was found in the sample relative to sucrose with a total sugar of 22.689 mg/l. A decrease in variation of sugars in 2010 winter season was observed to be higher than the concentration of sugars in 2011 winter season, this variation were attributed to the intensity of the winter season in 2011 compared to the 2010 season.
During the 2010 spring time; higher variation in sugar concentration was observed, were the total absence of sugars of about -14.602 mg/l relative to glucose was indicated in the sample while a negligible amount of total sugars of about 0.666 mg/l was observed, higher increase in sugar relative to sucrose was higher with 15.268 mg/l. This could be attributed to a prolonged winter season of the year. In 2011summer season, the sugar concentration in the sample increased by 42.851 mg/l and 44.373 mg/l respectively, while the total concentration of sugars in the sample increased to about 87.229 mg/l.
From the result presented in Table 3, it was observed that the mass of glucose in the sample biomass has a direct proportional relationship with the concentration of sugars in the sample and average weight of the biomass. Table 3 shows in July 2009 that the initial mass of biomass of about 3876mg/kg relative to glucose yield glucose concentration of 41mg/l while in sucrose 241mg/l of biomass is produced by 2.566mg/l concentration of the sugar. This shows that as the mass of sugars in the biomass increases the concentration also increases and decreases when the mass of the sugars in the biomass decreases.

Table 3. The concentration of sugars in dry weight of the sample biomass

Time (month)	Average-Absorbance of the sample (nm)	Concentration of glucose in solution (mg/l)	Concentration of sucrose in solution (mg/l)	Dry weight of biomass (g)	Total concentration of sugars in the sample (mg/l)	Average weight of glucose in biomass (mg/kg)	Average weight of sucrose in biomass (mg/kg)	Total sugars in the biomass (mg/kg)
Jul-09	0.264	41.322	2.566	0.0533	43.888	3876.	241	4117
Aug-09	0	0	0	0	0	0	0	0
Sep-09	-0.049	-63.057	-9.5	0.0529	-72.557	-5960	-898	-6858
Oct-09	0.188	15.911	29.456	0.0515	45.367	1545	2671	4215
Nov-09	0.365	75.089	59.045	0.0527	134.134	7124	5602	12726
Dec-09	0.138	-0.557	21.222	0.0523	20.665	-53.	2029	1976
Jan-10	0.244	34.611	38.806	0.0536	73.417	3229	3620	6849
Feb-10	0.427	95.689	69.345	0.0522	165.034	9166	6642	15808
Mar-10	0.332	63.9	53.45	0.0517	117.35	6180	5169	11349
Apr-10	0.612	152.873	100.122	0.0528	252.995	14477	9481	23958
May10	0.749	197.917	122.943	0.0532	320.86	18601	11555	30156
Jun-10	0.267	39.309	42.583	0.0538	81.892	3653	3958	7611
Jul-10	0.094	-17.544	13.778	0.0536	-3.766	-1637	1285	-351
Aug-10	0.138	-3.081	21.106	0.0535	18.025	-288	1973	1685
Sep-10	0.131	-5.208	20.028	0.0547	14.82	-476	1831	1355
Oct-10	0.202	18.103	31.839	0.0524	49.942	1721	3038	4759
Nov-10	0.108	-12.851	16.156	0.0526	3.305	-1222	1536	314
Dec-10	0.243	31.502	38.628	0.0522	70.13	3017	3700	6717
Jan-11	0.075	-23.849	10.583	0.0522	-13.266	-2284	1014	3347
Feb-11	0.198	16.765	31.161	0.0519	47.926	1615	3002	4617
Mar-11	0.1	-15.318	14.906	0.0515	-0.412	-1487	1447	-40
Apr-11	0.129	-5.757	19.75	0.0525	13.993	-548	1881	1333
May11	0.078	-22.732	11.15	0.0528	-11.582	-2153	1056	-1097
Jun-11	0.278	42.851	44.378	0.0525	87.229	4081	4227	8308

According to the result observed during the spring season (April and May, 2010), the highest level of biomass 14477mg/kg and 18601mg/kg increased by 22% of glucose with 152.873mg/l and 197.917mg/l respectively, and the same to sucrose with a higher increase in biomass level while the total weight of

biomass in the plant is 30156mg/kg. It then shows that the mass of sugar deposited as a result of photosynthesis, significantly contributes to the weight of the biomass.
It was also observed that high variations of sugar relative to sucrose were identified from early-summer (June 2010 to June 2011), a comparison of these results showed that about 10% decrease in weight of biomass was observed in glucose during the summer season compared to the 6% decrease in weight of biomass relative to sucrose. This signified that the amount of sucrose deposited in the plant cell during summer is higher compared to the amount of glucose (Table 3).
The lowest weight of the biomass was not suitably identified due to the high dilution of the sample, although based on the result it was observed from May 2010 to February 2011 with a decrease in sample biomass of about 18601mg/kg to1615mg/kg. The implication of this result is that; smaller the biomass, the most likely is it to have a lower mass of sugars present in the sample (*Laminaria digitata*), and hence likely the potentials of synthesizing bioethanol from the sample.
Conversely, a larger biomass shows the presence of more sugars which the macroalgae feeds on for its growth and development and hence the greater the potential for the synthesis of bioethanol from the sample with larger biomass (Table 3).

4. Conclusion

Sugar concentrations in analysed samples changed with time during the different months of sample collection. The result of wet chemistry analysis of the sample indicated different colours such as green, yellow and colourless solutions which signified the presence of carbohydrates. The green colours specifically represent glucose and sucrose while yellow and colourless solutions represent other unknown sugars and organic compounds. The standard curves obtained from the sucrose and glucose standards showed a linear relationship and direct proportionality between absorbance of light by the sample and the sugar concentration. There was an observed significant value of sugar concentrations in the samples denoting the presence and formation of sugars in the plant cell as a result of photosynthesis. The increase and decrease in sugar concentrations at different months reflects gain and loss of carbohydrates and is consistent with the measured absorbance at different months of the year. The carbohydrates observed were mainly glucose and sucrose and production rates were at the maximum during April 2010 and May 2010 with peak values of 152.873mg/l to 197.917mg/l and 100.122mg/l to 122.943mg/l for glucose and sucrose respectively. The period represents the spring season where optimisation of carbohydrate for bioethanol production is at the maximum. Other periods and seasons also showed different levels of sugar production which is a clear indication that bioethanol can be produced all year round, although from the results of the seasonal variations, the highest production can be achieved during the spring season when longer days, adequate rainfall and sunshine were favourable for growth of photosynthetic macroalgal cells and carbohydrate production. This is a clear indication that macroalgae are potential sources of carbohydrate for use in bioethanol fermentation. The carbohydrate sugars identified were glucose and sucrose whose standards were available whereas the other important sugars of interest such as Laminarin, zylose, mannose, arabinose and mannitol were anticipated to be present but could not be measured due to the unavailability of their individual standards and difficulty in communicating the HPLC equipment which would have been used to detect these sugars, thus using an alternative method. The concentrations of the total sugars had minimum value of 320.86mg/l and maximum value 3.305mg/l of respectively. The highest amount of total sugars was obtained in May 2010, during the spring season with about 230.401mg/l.
The mass of sugars deposited as a result of photosynthesis significantly contribute to the weight of the biomass. The implication of the result was that; the smaller the biomass, the most likely it was to have a lower mass of sugars and hence the less likely the potentials of synthesizing bioethanol from the sample. Conversely, a larger biomass signified the presence of more sugars (glucose and sucrose) and hence, the greater the potentials for synthesizing bioethanol from the samples with larger biomass.

5. Recommendations

Based on the present status of bioethanol production, further awareness is required to enlighten the global society on the benefit of macroalgae for bioethanol fermentation as well as its potentials for addressing the food versus fuel issues of the previous bioethanol production from terrestrial plants. Still unattended include, the national legislation and regulations for handling and using fuels which are at their initial phases, tax reductions as well as other incentives which could be necessary to make the fuel more competitive. Further research work is required in other areas such as the technical approach,

commercial demonstration. Also, funding should be encouraged by governments, multinational companies, public and private sectors.

It was discovered that though the analysis of carbohydrates through UV-visible spectrophotometer is simple and convenient to perform, however; it is limited in its ability to detect all the sugars in different concentrations as well as the sugar types, this could be as a result of the insensitivity of the absorption spectrum of the equipment to detect different sugars from the samples in all the concentrations used. Meanwhile, the use of high performance liquid chromatography (HPLC) is strongly recommended for this analysis because it could be used to detect all the carbohydrate types present in the samples as well as graphically indicate various peaks of the carbohydrate types at different periods. Although, this method could be quite expensive to operate on; the cost of training technical personnel to man the operations of the equipment is also high.

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