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Growth and Analysis of Algal species as a Source of Bio-diesel

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Algae are heterogeneous group of organism which live in damp habitats on land. In the present study, selected algal species are being screened for their suitability for bio-diesel production. Three species, *Scenedemus sp.*, *Spirogyra sp.*, and *Spirulina sp.* have positively been identified to the genus level and are being analyzed. pH range of 8.3 – 8.7 has been found to be necessary for the growth of *Scenedemus sp*, a medium with macro-nutrients and micro-nutrients has been developed for the growing of *Scenedemus sp.* .Also, the growing surface area need to be determined very carefully since it seems to be quite important in the growing of *Scenedemus sp*. *Spirogyra* was found to contain 2.18 ± 0.36 % fat, 20.83± 0.00% crude protein, 39.84 ± 0.84 % total carbohydrates, 6.30 ± 0.50 % fibre, 20.19 ± 0.62 % ash and 10.66 ± 0.41% moisture. The fat content was analyzed and found to contain octanoic acid, tetradecanoic acid, hexadec-9-enoic acid, octadecanoic acid, oleic acid, linoleic acid and linolenic acid. From the amount of oil content it can be concluded that the *spirogyra* can not be economic for bio-diesel production in its present form.

Key words: Algae, *Scenedemus sp., Spirogyra sp., Spirulina sp* and bio-diesel

Introduction

The algae that are used in biodiesel production are usually aquatic unicellular green algae. Under good condition green algae can double its biomas in less than 24 hours (Christi, 2007; Schneider, 2006). This high yield, high-density biomass is ideal for intensive agriculture.

The water used in algae cultivation can be fresh or saline, and salt concentration up to twice that of sea water can be used effectively (Brown and Zeiler, 1993; Aresta *et al*., 2005). This means that algae need not compete with other users of fresh water. Algae also has a greater capacity to absorb CO_2 than land plants, and are also prone to photosynthetic inhibition under conditions of intense of sun light (Brown and Zeiler, 1993).

Through a combination of this light water and carbon fertilization techniques the production of high density algae is starting to be achieved. Two experimental facilities, the Oakridge National Laboratory yields 60 gm^{-2} $d^{-1}$of algae and the ASP Red Hawk

Power plant yields algae with an outstanding average of 98 gm^{-2} d^{-1}(Schneider, 2006; Pulz, 2007).

Materials and Methods

Samples of *Scenedemus sp* were cultured in the green house while *Spirogyra sp* was sampled from stagnant water a long Narok river.

P^H measurements

The P^H of the growing media was varied with the introduction of $NaHCO_3$ as source of CO_2 .The P^H was measured using Hanna P^H 211 microprocessor pH meter.

Growth nutrients

Two types of growing nutrients were prepared and labeled as foliar A and foliar B. Foliar A consisted macro-nutrients and micro nutrients while foliar B consisted only macro-nutrients and their growth was monitored using hemacytometer and Kruss optronic GmbH light microscopy model MBL 2000.

Foliar A had 20% N, 20% P, 20% K and 2% Mg as macro-nutrients and 225ppm Zn, 125ppm Cu, 55ppm Mo, 400ppm Fe, 320ppm Mn and 55ppm B. Foliar B had 15% N, 12% P, and 7% K as macro-nutrients.

Moisture Content

A quantity of 2g of well-mixed sample was accurately weighed into a moisture dish and transferred to an air oven previously heated to temperatures of 130°C and drying done for 1 hour. The final weight of the sample was taken after the drying period and cooling in a desiccator. The loss in weight was reported as moisture (AOAC, 1995).

Crude Protein content

A quantity of 0.3g of sample was weighed into a digestion flask and 2.5ml of 3.2g salicylic acid added and mixture was added into 100ml of concentrated sulphuric acid – selenium mixture. The mixture was heated in a fume hood at 110ºC for 1hour. After cooling, three successive 1ml of hydrogen peroxide were added and the temperature rose to 330ºC. The digest was cooled, transferred to a 50 ml volumetric flask and topped up to the mark with distilled water. A blank digestion with the catalysts and acid was also made. 5ml of diluted digest was transferred into the distilling flask and washed with 2ml distilled water. 15 ml of 40% NaOH was added and this was also washed with 2ml distilled water. Distillation was done to a volume of 60ml distillate. The distillate was titrated using 0.014N-HCl to an orange colour of the mixed indicator which signified the end point as describe by (Okalebo, 2002).

Crude Fat Content

The Soxhlet extraction method which gives intermittent extraction of oil with excess of fresh condensed organic solvent was used. Fat extraction was done using hexane for 16 hours. The extraction solvent was evaporated and the extracted fat dried in a hot air oven for 15 min (AOAC, 1995)

Ash Content

Sample weights of 5g were measured in pre-conditioned crucibles. The samples were first charred by flame to eliminate smoking before being incinerated at 550°C in a muffle furnace to the point of white ash. The residues were cooled in desiccators and the weights taken (AOAC, 1995)

Crude fibre

A quantity of 2g of sample was weighed into a 500ml conical flask. 200ml of boiling 1.25% H_2SO_4 was added and boiling done for 30min under reflux condenser. Filtration was done under slight vacuum with Pyrex glass filter (crucible type) and the residue washed to completely remove the acid with boiling water. 200ml of boiling 1.25% NaOH was added to the washed residue and boiling done under reflux for another 30 min. Filtration was done using the same glass filter previously used with the acid. The residue was rinsed with boiling water followed by 1% HCl and again washed with boiling water to rinse the acid from the residue. The residue was washed twice with alcohol and thrice with ether and then incinerate for 3 hours (AOAC, 1995).

Total Carbohydrate

Under this approach, the other constituents (protein, fat, moisture, fibre, ash) were determined individually, summed up and subtracted from the total weight of the sample is referred to as total carbohydrate by difference(FAO, 2003).

Figure 1 Shows the green house where the scedesmus sp and spirulina sp is grown

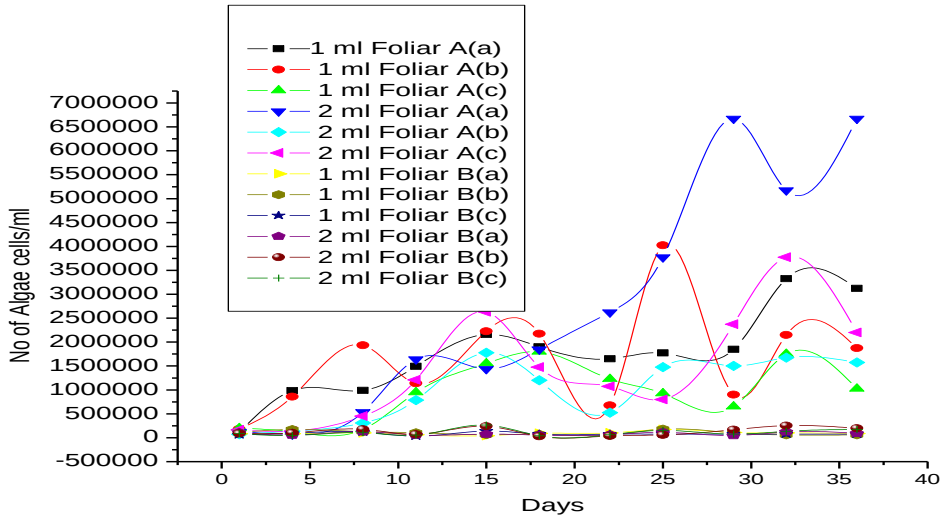
Figure 2: *Scenedemus sp* growing in the volumetric flasks

Figure 3: S*pirulina sp* growing in a pond in the green house

Results and Discussions

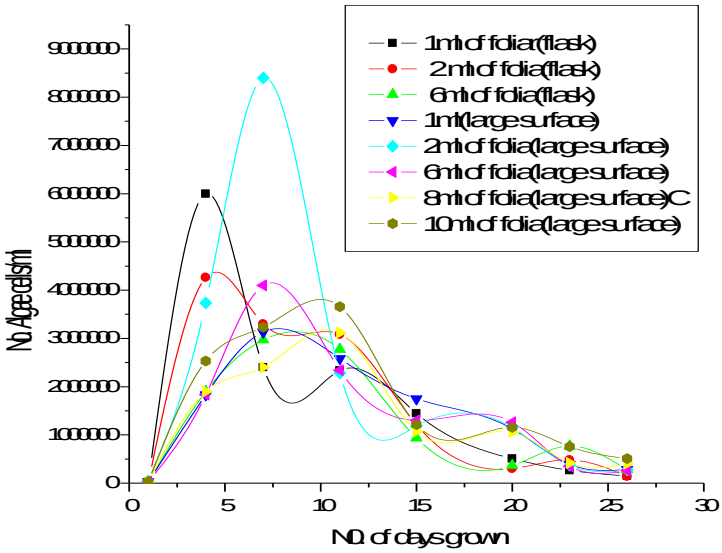
From graph 1, it can be seen clearly that foliar A which contained both macro-nutrients and micro-nutrients had a higher growth for the entire growing time of the *Scenedemus sp* as compared to foliar B which its growth was fairly constant for the entire growth period. Maybe the foliar B macro-nutrients were enough for maintaining the survival of the *Scenedemus sp.*

From graph 2, a concentration of 2ml of foliar A and large container favoured the growth of *Scenedemus sp* and the highest growth of approximately 9 million cells/ml was recorded after seven days of growth, while the same concentration of foliar but in a 500ml flask had the highest growth of approximately 6 million cells/ml recorded after 5 days of growth. In general, we can conclude that a large surface is needed for maximum algal growth. From graph 2, it can also be noted that there needs to be the right amount of foliar A for a maximum growth to be achieved since these can be demonstrated by the concentrations of 1ml, 6ml, 8ml and 10ml of foliar A did not lead to such high growth but it was also noted that the *Scenedemus sp* had some growth but not as pronounced as when it was right amount of 2ml of foliar A.



Graph 1: Foliar A against foliar B in growing of *Scenedemus sp*

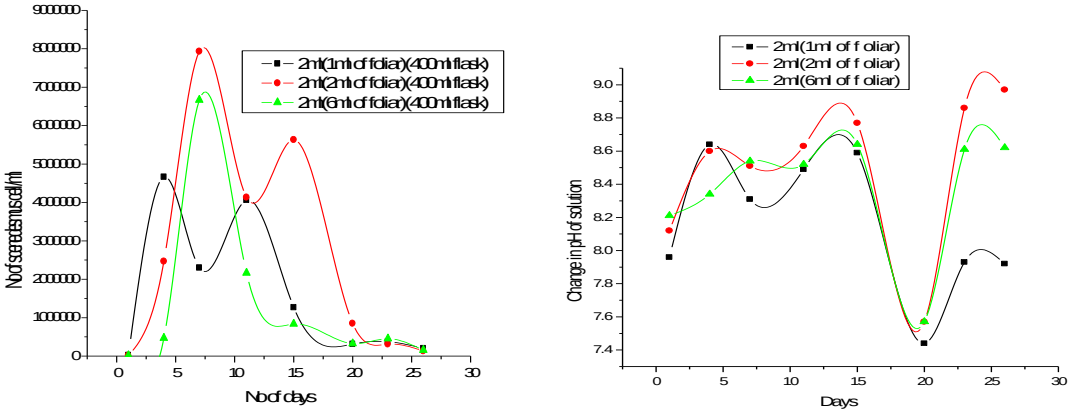
From graph 2 , a concentration of 2 ml of foliar A and a large surface area had the highest growth after 7 days with approximately 9 million of *Scenedesmus sp.* cells while same concentration in a small flask had their highest concentration of about 6million after 5 days. It can also be noted that the large surface is one of the factors influencing the growth of algae. The maximum growth of this species could be noted to be within the first 10 days there after the number of cells decreased gradually.



A graph showing growing of 1ml of scenedesmus sp in different foliar concentration

From graph 3 above, it was noted that the pH is a main factor in the growth of scenedesmus sp. The pH in the range of 8.3-8.7 units favors the growth scenedesmus sp. Decline in growth was observed as the as the pH decreases. Even though the pH increased after twentieth day the growth was significant. This could have been due exhaustion of nutrient or the surface area had been saturated hence could be coagulate and sedimentation taking place.

Graph (a) shows growth of scenedesmus sp when different when different concentration foliar applied and graph (b) shows the change in pH during same period

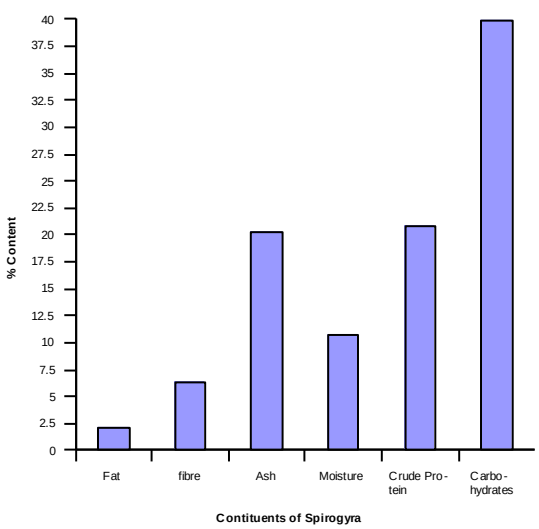


Conclusion

P^H range of 8.3 – 8.7 is necessary for the first 10 days of *Scenedesmus sp* growing period. When the pH is in this range after 10 days in a small surface area then it does not favor the growth. Its also been noted that growing of *Scenedesmus sp* depends greatly on the surface area hence the growing area has to be determined carefully to maximize yield, otherwise the cells will coagulate and finally sediment. Finally, *Spirogyra sp* with fat content of 2.18% is quite low for it to be used as a source of bio-diesel unless it is genetically modified to produce more lipids as compared to protein or total carbohydrates.

Table 1 and Graph 4 The constituents of *Spirogyra sp*

Constituents	Content
Fat	2.18 ± 0.36
fiber	6.30 ± 0.50
Ash	20.19 ± 0.62
moisture	10.66 ± 0.41
crude protein	20.83 ± 0.00
Carbohydrates	39.84 ± 0.00



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