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Abstract:

All over the world fossil fuels are used every day for power production. Fossil fuels can be easily found across the globe. But the quantity of these fuels are limited, and is estimated to end in the future. Microalgae are one of the most promising sustainable source of lipids as a key alternative to petroleum liquid fuels and first-generation biodiesel, believed to be capable of meeting global transport fuel needs**.** This study focused upon isolation and selection of local microalgae species and scaling up of the suitable species at outdoor condition for biomass production to extract biodiesel. Two different species were isolated, Scenedesmus and light green colored Chlorella. Scenedesmus and light green colored Chlorella were found to have specific growth rate of 0.23 day-1 , 0.19 day-1 respectively whereas the dry biomass production were 0.708 mg/ml and 0.555 mg/ml respectively. Scenedesmus and light green colored chlorella were chosen for additional large-scale testing for biodiesel production at outdoor condition. It demonstrated relative productivity and biomass accumulation as compared with traditional photobioreactors. This species also displayed good tolerance, as there was no temperature and light regulation. Such wild species, being in outdoor condition, also showed strong tolerance in temperature fluctuations. Oil was obtained from liquid enrichment culture 0.30ml / gm (dry weight) using direct transesterification method

Keywords: Scenedesmus, Chlorella, biodiesel, microalgae, photo bioreactors, dry-biomass,fossil fuels.

Introduction:

The exhaustion of non-renewable energy resources on the Earth has initiated researchers to explore novel and alternative energy sources. Due to the concern of energy crisis and the negative environmental effects of burning fossil fuels, renewable energy with low level of carbon dioxide emission are highly explored in recent years. Sustainable and renewable energy sources such as hydroelectricity, solar energy, wind energy, wave power, geothermal energy and tidal power are able to generate clean electricity. Renewable biodiesel has, of late, attracted some global interest, mostly due to for their immense economic and environmental gains. Biodiesel is Biodegradable, non-toxic and capable of reducing the level of air pollution by reducing the greenhouse gas emissions to the atmosphere. Biodiesel can be divided into three generations: first-generation biodiesel is extracted from edible oil (e.g. rapeseed oil palm oil and soybean oil), second-generation biodiesel is mostly generated from non-edible oil (e.g. jatropha oil, pongamia pinnata oil and frying oil), and third-generation biodiesel is synthesized from microbial lipid**.** There are many first-generation biodiesel disadvantages, such as competition with food supplies, large arable land requirements, high water and fertilizer demand, deforestation, and loss of biodiversity. Researchers therefore strongly recommend biodiesel of the second generation from non-edible oil sources, but the feedstock is still small for commercial exploitation.

Microalgae have recently been highlighted as the most feasible feedstock for 3rd generation biodiesel production. Microalgae are a group of microorganisms whose cellular structure is simple. Microalgae are capable of producing and storing triacylglycerol's (TAGs) in their cells , especially in the stress environment. In these circumstances, microalgae will cease to divide and the TGAs will be stored in their cells as a way of survival to withstand such adverse conditions. TAGs typically take the form of storage lipids in the cell and can be converted to biodiesel.

Microalgae have lately become a major feedstock for biodiesel Development, within their cells due to their high lipid content, and they can be Fast replicated. Microalgae are typically a single cell , so they can acclimatize to General requirements of the world and thus flourish. Additionally, Experts have discovered more than 40,000 different types of microalgae that contain 20 to 50 percent of the lipid content of their total biomass, a good feature of biodiesel generation.

Microalgae may be heterotrophic or autotrophic. For the production of cells, autotrophic microalgae need only inorganic components such as CO2, salts and sunlight; while heterotrophic need extra source of organic components and nutrients as an energy source. Microalgae are prokaryotic or eukaryotic photosynthetic microorganisms that can live under harsh condition due to their simple cellular structure

Microalgae can generate various types of renewable biofuels, such as, the production of bio methane by anaerobic digestion, bio hydrogen by photo biological process under certain conditions and biodiesel by transesterification process.

Objective:

The goal of this research is to develop microalgae for biofuel production. To achieve the target, the following objectives were put out:

- Isolation and scale up of specific single local strain suitable for the production of biofuels
- Experiment to conduct batch lab scale growth.
- Examining the impact of aeration on the growth rate to obtain adequate mass for the production of biofuel
- Examining the algal sample, to find the oil content using a Direct Transesterification Process.

Methodology:

One batch of microalgae sample was collected from stagnant water found in our native pond in front of the IICT Building, Shahjalal University of Science and Technology, Sylhet area, Sylhet. It was primarily taken into a PET bottle and transported into the laboratory as soon as possible for further processing. The sample was observed under microscope to ensure that if it contains microalgae.

The collected sample was at first enriched in BG-11 broth to preserve the dominant species and to ensure that the medium is suitable with the species. Inoculated species with ration 1:9. 10 ml of the sample was directly inoculated into a 250 ml conical flask with 90 ml autoclaved sterilized liquid solution. This meant the algae did not have to compete in the medium for nutrients with native bacteria. Half a dozen of conical flasks were held on the side of a window under direct sunlight to envision development by naked eyes with respect to the blank media. For enabling aeration, the culture broth was shaken manually for three to four times a day, and no forced air was given for the

ones kept in direct Sunlight

To achieve a single strain, the enriched culture sample was spread and streaked onto petri dishes containing BG-11 solid media. The Petri dishes were sterilized by keeping for 1 hour at 180 ° C in autoclave. At first these plates were allowed to cool, then held inverted for non-drying and then prepared for spreading or stretching

8 ml direct sample was taken and centrifuged at 5000 rpm for 5 minutes. Supernatant was discarded and pellet was washed repeatedly using vortex mixer with distilled water. 2 ml inoculums were added on to the solidified media by spreading method. The enriched culture samples were spread on BG-11 agar plates without any centrifugation but using serial dilution technique

The microalgae obtain from direct sample were initially washed of dirt and foreign particles and then allowed to streak through the loop in agar plate and incubated at the same condition. Streak-plating were repeated for at least two times to pick up a single colony after growing in test tubes.

Single colonies were picked up by needle loop and allowed to grow in test tubes containing 5 ml liquid medium for 1 week. Before putting in the tubes and vials, the single cell growth and purity of single species will be confirmed after observing under microscope.
After growing into the conical flask it is finally diluted with 450 ml liquid medium providing with aeration and kept into direct sunlight at outdoor condition.

Microalgae identification was performed using morphological taxonomic keys found in – A beginner's guide to freshwater algae. A drop of liquid culture from test tube was kept on glass slide and cover slip was placed over. The identification of the algal cultures was done by observing under the compound microscope (40x) up to genera level. The morphological characters considered for identification were cell structure, color, shape etc.

Algal biomass must be harvested during exponential phase before reaching stationary or lag phase. Harvesting in this case was done at 15th day.

Algae would grow optimally when the nutrients and the light source are sufficient. Many researchers use OD reading to monitor the growth of algae and the wavelength used by researchers vary depends upon species of algae, growth conditions, and growth measurements. There is not a standard wavelength for measuring OD with algae, it can vary from around 500nm to 700nm.

A dry and clean falcon tube was weighted first and recorded for measurement of wet and dry biomass. Samples were taken from 15 ml of falcon tube. It was then centrifuged for 8 minutes at 5000 rpm. Supernatant was carefully discarded, and measured again. The difference of this two weights was measured in wet mass. This falcon tube was then dried for 24 hours at 85 °C and measured again and calculated dry mass

2.2 gm dry microalgae was grind in a mortar pestle. 7.48 ml methanol, 1.32 ml sulfuric acid, and 4.4 ml of chloroform was added with it. It was then placed into a magnetic stirrer at 90^0C temperature for 45 minutes which provided thorough mixing. It was then again cooled to room temperature. 4 ml of distilled water was again added and again mixed for 60 seconds. Separation in phase was allowed. Water was then again applied to the oil mixture, and the oil floated on water.

Result & Discussion:

Initial growth experiments were conducted by the condition said above in liquid medium. After three-four days the medium turns into greenish and at day 7 noticeable growth was observed (Figure 1.1). The test showed that the sample algae did survive well in the selected medium.

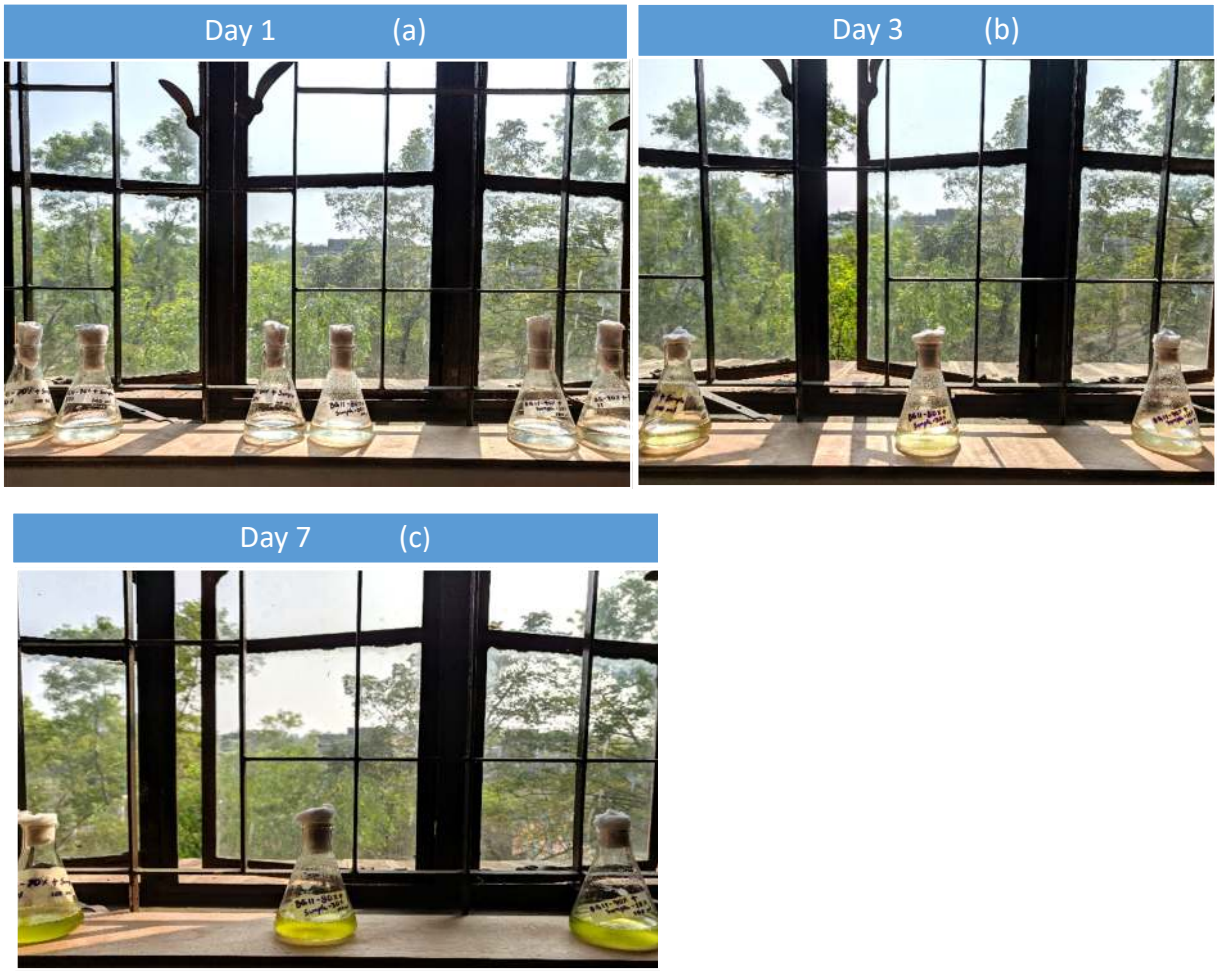
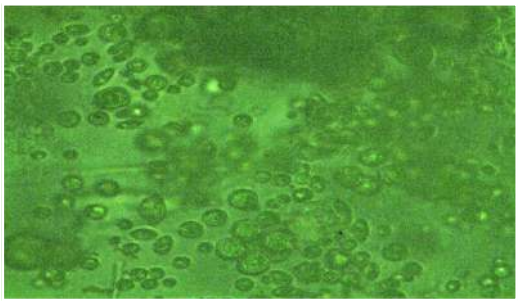


Figure 1.1 : Liquid Enrichment of sample in BG11 broth media (a) Transparent at 1st day(b) Light green color appears at 3rd day (c) Turn into visible green on 7th day

From microscopic view **Figure 1.2** initially two different type of species were observed. A good number of small spherical dark green colored cell were observed. Two or four cells were clustered together as well as single cells were also present. These were similar to chlorella. From literature review it was found that the oil content (% dry weight) of various species of chlorella varies from 5-58%. The lipid productivity accounts around 11.2-40 mg/L/day.

A fair number of oval shaped 4-8 cells were arranged in a row. These characteristics are as similar as scenedesmus. The oil content of (% dry weight) of various species varies from 40- 75%. Both of this two species are frequently abundant in nutrient rich water. From microscopic view it is seen that both are well suited in the medium.

Fig : 1.2 Microscopic view of liquid culture at 40X zoom



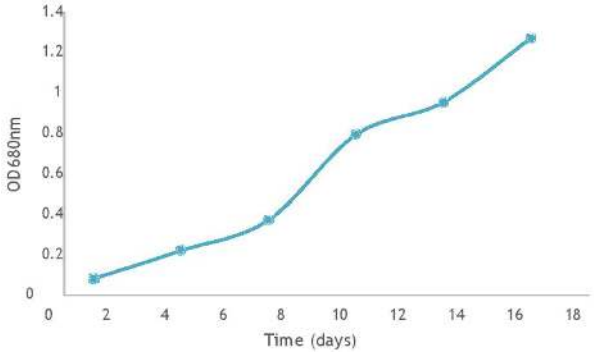


Fig 1.3: Optical density profile for Scenedesmus at outdoor condition

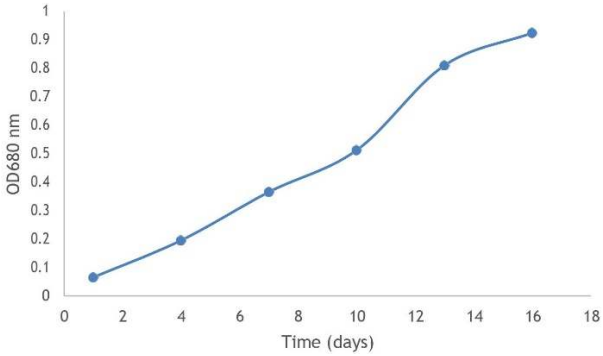


Fig 1.4: Optical density profile for light green Chlorella sp. at outdoor conditions

After direct transesterification process the algae biomass turned into a substance like black paste and no visual presence of oil was found. Water was added into it for quick assay of oil and a layer was float on the water.

The amount of oil was nearly 0.3 ml obtained from 1.05 gm dry algae sample. The amount was insufficient for separation and further characterization

Conclusion:

In Bangladesh perspective the idea of using algae as a fuel source is relatively new and very few researches were conducted in this field. A very few numbers of published papers were found that had reported oil production, however all of those reported species were either of foreign origin or tested in conventional photo bioreactor system which is costly. The main goal of this theses was to identify and isolate a high lipid containing strain from local sources for biodiesel production rather than collecting it from other countries

Acknowledgement:

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References:

Andrich, G.; Nesti, U.; Venturi, F.; Zinnai, A. & Fiorentini, R. (2005). Supercritical fluid extraction of bioactive lipids from the microalga Nannochloropsis sp.. European Journal of Lipid Science and Technology, Vol.107, No.6, pp. 381-386

Xinbei Tan, Yoshimitsu Uemura, Jun Wei Lim, Chung Yiin Wong, Keat Teong Lee, Cultivation of microalgae for biodiesel production: A review on pstream and downstream processing, (2017)