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Assessment of the Growth Kinetics of Selected Microalgae Species in Synthetic Wastewater for the Production of Biodiesel

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Abstract: The need to reduce the dependence on the petroleum based fuel is becoming important because of the global warming and also due to energy crisis. Continuous combustion of fossil carbon has an effect on increasing the amount of greenhouse gases (GHG). Biodiesel is one of the substitutions of fossil fuels. Production of biodiesel from microalgae is categorized under third generation biofuel which is considered to be a viable alternative energy resource. This study focused on investigating growth kinetics of microalgae, cultivated under laboratory conditions. Three freshwater microalgae species were isolated, from the water samples collected from two reservoirs included Victoria and Ulhitiya, representing only the Intermediate climatic Zone of Sri Lanka. Algae species were cultivated in a synthetic wastewater medium followed by a kinetic study, in order to identify the species with the highest potential for the production of biofuel. Light intensity, aeration and temperature were varied as the physical parameters during the experiment. According to the statistical analysis, *Chlorella* sp. showed highest growth rate than *Monoraphidium* sp. and *Scenedesmus* sp. in all three conditions.

Keywords: Biodiesel, Microalgae, Growth kinetics, Wastewater

1. Introduction

The importance of using renewable green energies is being debated globally since it is understood the harmful effects due to continuous combustion of fossil fuel. These concerns have increased the interest in developing first and second generation biofuel, such as vegetable oils and lignocelluloses [20]. Due to various issues occurred in previous generations of biodiesel, scientists are now aiming for third generation of biodiesel, where the microalgae and sea weeds could be used as the energy source. The idea of producing methane gas from algae was not recent and goes back to early 1950s. Also the oil crisis of the 1970s resulted in algal research during the period of late 1970 to early 1980 [4]. Nevertheless, due to the abundance of fossil fuel, the concept may have not come to reality. However due to the scarcity of energy sources such theories are now experiment to find sustainable solution for the current energy crisis [21].

Microalgae have a high potential as biodiesel precursors because many of them are very rich in oils. It has been discovered that the average lipid content of microalgae varies from 1%-70% of dry weight [14]. Triacylglycerides (TAGs) generally serve as energy storage in microalgae and once extracted can be easily converted into biodiesel through transesterification.

Lipids production and extraction from algae depend on algal species and extraction solvent system.

The unique character of microalgae is, many species of microalgae are able to effectively grow in wastewater conditions because of their ability to utilize abundant organic carbon and inorganic N and P in wastewater [5]; which provide a co-benefit of producing biofuels. Therefore there is a potential of combining the goals of sustainable fuel production and wastewater treatment by cultivating microalgae in wastewater. In addition, the cultivation of microalgae in wastewater offers a far more attractive proposition from an environmental point of view, specifically for industrialized and developing countries.

Cultivation of microalgae is being done throughout the world for the production of biodiesel and also as a wastewater treatment process, but albeit on a relatively minor scale [1, 5]. In the commercial scale of microalgae cultivation, it has become important to concern on growth rates of the algae species in a condition. Therefore kinetic study of the microalgae biomass is an important parameter for determining its suitability for commercial or large scale mass production for the extraction of oil and other useful compounds [16].

This work aimed to evaluate the laboratory cultivation of three selected microalgae species isolated from Sri Lankan reservoirs. The Species were cultivated by varying physical parameters in order to find the optimum conditions for each species

2. Materials and Methodology

2.1. Algae Sample Collection

Algae samples were collected from two selected reservoirs in Sri Lanka, Victoria reservoir in Central province (7° 14' N, 80° 47' E) and Ulhitiya reservoir in Uva province (7° 27' N, 81° 3' E) during the months of July and August, 2014.

Water samples for culturing of algae were collected using plankton nets with the pore size of 34µm. And on site measurements for temperature and conductivity were taken from each sampling site during the time of sample collection. The collected algae containing water samples were preserved in a cool pack and taken in to the laboratory.

2.2. Cultivation Methods

For the cultivation of microalgae, a synthetic wastewater medium was used as the culture medium. The culture media was prepared using the method outlined by the formulation of local wastewater treatment plant designed guideline.

2.2.1. Plate Culturing

An amount of 200 ml was taken from the prepared culture medium and 2.00g of agar was added. The culture medium and other required glassware were autoclaved under pressure of 15 psi, temperature (121°C), for about 15-20 minutes. The autoclaved medium was transferred into petri dishes /culture plates under laminar flow cabinet and the plates were covered until the agar medium solidifies.

A dilution series was prepared from 10⁻¹ to 10⁻³, by using algae containing water samples which were collected from the reservoirs. An amount of 100 µL was taken from the samples with a dilution factor of 10⁻³ and was transferred on to the solidified culture medium. The sample was spread with the help of sterilized glass spreader. The edges of the culture plates were sealed with parafilm and allowed to incubate under natural conditions for 4-10 days.

2.2.2. Isolation and Cultivation of Algae Species

After the establishment of algal colonies (~ 10 days), using a sterilized loop each colony was transferred in to 100 ml of culture medium. The algae inoculums were kept for 10-14 days under natural conditions. After the incubation period, three pure algae cultures were obtained. The cultures were morphologically identified

as *Chlorella* sp., *Monoraphidium* sp. and *Scenedesmus* sp. The identified three algae species were cultivated in 10L containers using synthetic wastewater as the medium, until the kinetic study was carried out. An amount of 10 ml from each algal inoculum were transferred into 250 ml conical flasks containing the culture medium three weeks prior to kinetic study. This was done in order to obtain well grown and healthy algal cells.

2.3. Kinetic Study

2.3.1. Inoculation Ingredients

Cultivation of microalgae species for the kinetic study was carried out in 750 ml conical flasks. At the beginning, 5 ml of algal solution were added to container. The remaining was filled with 295 ml of liquid culture medium to make the total volume as 300 ml. Light intensity, aeration and temperature were used as the growth variables for the inoculums. The kinetic study was carried out by varying the growth variables.

2.3.2. Growth Variables

Fluorescent bulbs (Orange Electronic, Day light, Sri Lanka) with different intensities were used to provide necessary light for photosynthesis to occur. Ambient/aquarium air pumps (EJET aquarium pump, 168) were used to provide aeration in different rates and the temperatures were varied with the use of thermostatic water baths (YCW – 01, Gemmy Industrial Corp, Taiwan). Table 1 show the rates and the quantities of the growth variables which were used in the kinetic study.

Table 1: Growth variables

Variable	Light intensity/Klux	Aeration/(cm ³ /min)	Temperature / °C
Light intensity	0.71	1	27
	1.77	1	27
	3.92	1	27
Aeration	0.35	2	27
	0.35	4	27
	0.35	8	27
Temperature	0.35	1	30
	0.35	1	35
	0.35	1	40
Control	0.35	1	27

2.3.3. Quantifying Growth Kinetics

There are multiple ways to quantify the growth of microorganism. However, in this study cell counting and optical density of algae were used to develop a kinetic model. The three algae species were grown under the conditions shown in the Table1. Growth kinetics of the algae species were calculated using the following equation.

$$\mu_{max} = \frac{\ln(x) - \ln(x_0)}{dt}$$

2.3.3.1. Monitoring and analysis of Algae Culture

Sample collection was done once per 5 days after introducing the algae species into the inoculum, for a period of 20 days. Daily monitoring of temperature (T), light intensity (L) with the use of lux meter (EXTECH, wide range light meter) and aeration (AE) was done to ensure the proper growth of microalgae.

2.3.3.2. Cell Counting

Microscopic inspection of microalgae is an essential component in cultivating and maintaining monocultures. Research microscope (Carl Zeiss, GmbH, Konigsallee, 9-21, Germany) was used for cell counting. A sedgewick rafter cell (PYSER – SGI Limited, United Kingdom) was used to count the number of cells.

2.3.3.3. Measurement of Chlorophyll

Chlorophyll A level of each sample was measured every 5 days using a spectrophotometer (Hach, DR 6000).

2.4. Statistics for Analysis

The cultivation of microalgae in different conditions was done to identify the best candidate for Biofuel production. All the statistical analysis was performed using the computer software Minitab (v.14) SYSTAT (v. 12).

3. Results

Results of the experiment reveal that the growth rates of the three algae species used in the experiment showed a significant difference in their growth rates depending on the growth conditions.

3.1. Algae Growth Rates with Light Intensity

All three species showed the highest growth rate at the intensity of 0.71Klux. *Chlorella* sp. showed the highest growth rate closely followed by *Scenedesmus* sp., where *Monoraphidium* sp. Showed the lowest growth rate (Figure 1)

It is clear that when the light intensity increases the growth rates of the algae species decrease. However at the intensity of 1.77 and 3.92 Klux, the highest growth rate was shown by *Chlorella* sp, where the lowest growth rate was shown by *Monoraphidium* sp.

3.2. Algae Growth Rates with Aeration

The growth patterns with aeration exhibit an increasing trend along the positive gradient of aeration (Figure 2). *Chlorella* sp. gave the highest growth rate among the three species, at all three aeration rates.

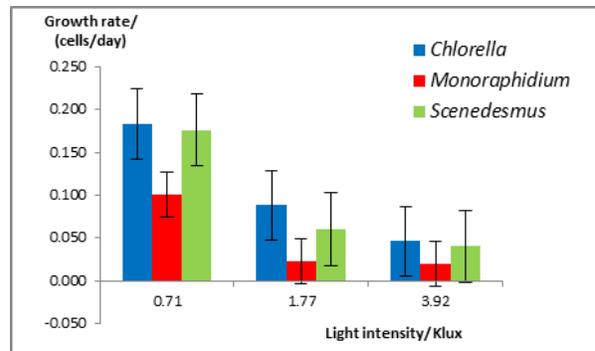


Figure 1. Growth rates of algae species at different light intensities

In advance it showed the highest growth rate at the aeration rate of 8cm³/min, and the lowest growth rate at the aeration rate of 4cm³/min. *Scenedesmus* sp. showed the highest growth rate at the aeration rate of 2cm³/min and the growth rate was slightly decreased as the aeration rate was increased. In contrast, while the other two species showed a noticeable difference in their growth rates, *Monoraphidium* sp. did not show a significant difference of its growth rate at all three aeration rates.

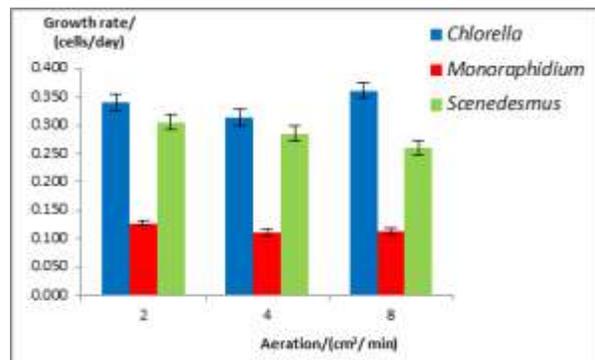


Figure 2. Growth rates of algae species at different aeration rates

3.3. Algae Growth Rates with Temperature

Temperature is another growth factor for microalgae. Theoretically the growth rate should be increased as the temperature increased. But in our study we noticed that the effect of temperature on microalgae varied depending on the algal species.

The Figure 3 illustrates the effect of temperature on growth rate of the three algae species. *Chlorella* sp. showed the highest growth rate at 30 and 35°C, whereas the lowest growth rate was shown by *Monoraphidium* sp. However, all three algae species indicate an overall negative growth rate at 40°C. Nevertheless, we were able to observe a quick growth of the three species within the first few days and with the time growth rates decreased.

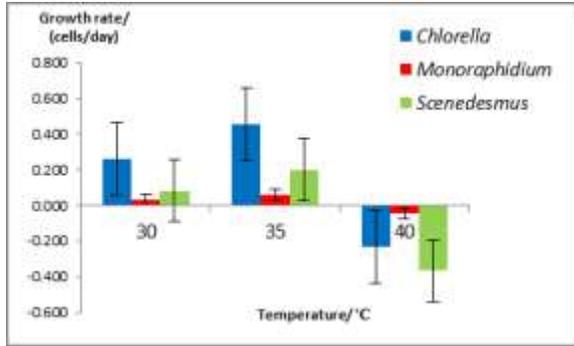


Figure 3. Growth rates of algae species at different Temperatures

According to the results obtained the optimum conditions for the growth of three microalgae species are summarized in Table 1.

Table 1: Optimum growth conditions for the algae species in laboratory scale

Species	Light intensity/ Klux			Aeration/ (cm ³ /min)			Temperature/ °C		
<i>Chlorella</i>	0.71	1.77	3.92	2	4	8	30	35	40
<i>Monoraphidium</i>	0.71	1.77	3.92	2	4	8	30	35	40
<i>Scenedesmus</i>	0.71	1.77	3.92	2	4	8	30	35	40

Table 1 indicates that, the three algae species almost have the same optimum conditions except *Chlorella* sp., which shows the optimum growth rate at the aeration rate of 8cm³/min, while the other two species show their optimum rate at the aeration rate of 2cm³/min.

4. Discussion

The two reservoirs which were used for the collection of algae samples are situated in areas with high potential and pollution influx. Therefore it can be assumed that *Chlorella* sp., *Monoraphidium* sp., and *Scenedesmus* sp., isolated have a high tolerance to be grown in polluted water bodies. In addition when consider the distribution of algal species in limnetic water bodies of Sri Lanka, *Botryococcus braunii*, *Monoraphidium* sp. and *Scenedesmus* sp. are widely distributed and highly abundant in polluted water. Therefore it is justifiable, why these species exist in Victoria and Ulhitiya reservoirs. According to previous studies, it has proved that algal genera *Chlorella*, *Monoraphidium* and *Scenedesmus* contain a considerable amount of lipid [19]. Therefore it is obvious that the isolated algal genera have high potential for biofuel production. As one objective of this research is to identify and cultivate algal species to treat wastewater, the algae with high tolerance to pollution should be good candidates to serve the purpose. Further cultivation process also can be improved by providing other optimum environmental conditions such as temperature and oxygen. Thus, as the

first step a synthetic wastewater medium was used for the growth kinetic study of the isolated algae species.

According to our growth kinetic study three species of algae showed a significantly different growth rates to varying light intensity, aeration and temperature. For example the reduction of the growth rates with the increment of the light intensity (Figure 1) can be related to photooxidation process. The photooxidation takes place, when cells are exposed to high light intensity in the presence of high oxygen concentration. As we used 750 ml container for the cultivation, during the photosynthesis process the container may get saturated with oxygen. As a result the photooxidative effect could promote photooxidative death of microalgae.

When consider the growth rate with aeration, *Chlorella* sp. showed highest growth rate, where *Monoraphidium* sp. showed the lowest growth rate. Therefore it was apparent that the aeration rates do not affect much on the growth rate of *Monoraphidium* sp. The main purpose of aeration is to supply the CO₂ and to maintain a homogenized culture medium. *Scenedesmus* sp. showed a slight reduction of its growth rate with the increment of the aeration rate. When CO₂ amount increases, pH can be reduced in the presence of water, due to the formation of carbonic acid. Therefore the reduction of growth rate of *Scenedesmus* sp. could be related to the presence of carbonic acid. The situation may be related to the high sensitivity of *Scenedesmus* sp. to pH than the other two species. However as the interpretation of results are based on the microcosm experiments, the macrocosm results could be different as the media can be more exposed to the natural environment where the other environmental factors could have more effect on the growth of the algae [23].

Temperature is one of the important environmental variables that directly affect the algae growth. Temperature influences the physiological processors of organisms, including influences in cellular chemical composition, the uptake of nutrients, CO₂ fixation in photosynthetic organisms, and the growth rates. In our study the growth rate of algae species showed a significant difference at different temperatures. However, all three algae species showed a higher growth rate at 35°C, and a negative growth rate at 40°C. Generally optimal growth temperatures for most of the algae are in the range of 5–40°C [17]. However temperatures over 38°C growth inhibiting substances such as, oleic acid and mono unsaturated omega 9 fatty acid production could be increased in algae species, specially under laboratory conditions. In addition, under heat stress condition or with heat shock, the algal protein content will decrease and will produce abscisic acid (ABA), which is a stress hormone. stress hormone is a key factor in controlling own stream responses such

as growth and also gene expressions [5]. Therefore it is apparent that the algae species showed a negative growth rate as a result of stress hormone produced at 40°C. According to the statement of Powell, at 36°C, there is an increment of chemical composition in algae by 30% [34]. Therefore it is obvious that the algae species have higher growth rate at 35°C.

However we encountered some limitations in our experiment. As we carried out this experiment in laboratory scale, the algae species might have responded differently due to the sensitive to given conditions than in the natural environment. In addition due to the small sample size there can be limitations in statistical applications. Furthermore, fluorescent bulbs were used as the light source, where the controlled temperature may also get affected by the heat emitted by the bulbs. As the counting of algal cells was also done manually, human errors could affect the final results. Although we used mono cultures of algae species, contaminations could be occurred in algae cultures during the experiment period.

5. Conclusion

This experiment illustrates the ability of microalgae to adapt and survive under different physical conditions. Among three algae species *Chlorella* sp. shows the highest tolerance and the growth rate in each different conditions. In addition *Chlorella* sp. bare the highest amount of lipid content among three species (5.0 – 58.0 (% Dry Weight Biomass)). Therefore there is a benefit of producing biomass and providing a renewable and sustainable feedstock for biofuels and other applications (medicinal, food etc.). Even though *Chlorella* sp. has a higher growth rate when studying on life cycles, it has the shortest life cycle as the *Monoraphidium* sp. has the longest life cycle in given laboratory conditions.

Algae sample collection from other reservoirs from different regions of the country is recommended as future work. And also this experiment can be extended to pilot plant scale for further studies by varying more parameters.

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